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KAPPAPHYCUS SEAWEED IN THE PACIFIC: REVIEW OF INTRODUCTIONS AND FIELD TESTING PROPOSED QUARANTINE PROTOCOLS

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EXECUTIVE SUMMARY

The secretariat of the Pacific Community (SPC) commissioned the Institute of Marine Resources (IMR) of The University of the South Pacific to: 1) conduct a literature review of the introductions of the seaweed *Kappaphycus alvarezii* to Pacific Islands Countries and the current state of seaweed farming in these countries and 2) to field-test their proposed quarantine protocol for introducing *K. alvarezii* to new locations.

This seaweed, which is farmed for its carageenan content, has been introduced to Pacific Island countries between1977 and 2002. The industry has met with varied success. In Kiribati it became an important cash crop for both the rural and national economies. In Fiji progress has been erratic. After several successful growth trials funded by government, aid donors and private industry, *Kappaphycus* production was in short bursts interrupted by problems such as political instability, marketing problems and cyclones. From 1993 to 1997 the industry in Fiji ceased to operate. Since 1997, however, production has recommenced, increasing to 515 t in 2000 and declining thereafter as a result of poor internal marketing arrangements. Despite these problems, the industry has the potential to be an important income earner in rural Fiji. In the Solomon Islands, after growth trials in 1990, the industry almost became commercially viable but collapsed when the international buyers (*Marine Colloids* and *SSP*), based in Fiji, withdrew from the South Pacific. A second trial in the Solomons is currently underway with the first exports expected by the end of 2003. In the Republic of Marshall Islands, a second trial began in 2002 after initial growth trials in 1990 failed for various reasons. This seaweed industry is yet to develop to a commercial level in Cook Islands, Federated States of Micronesia, French Polynesia, Tonga, Samoa, Tuvalu and Vanuatu. There have been growth trials in these countries, however, and in some cases experimental farms have been established.

These developments have required that whole plants or cuttings of *Kappaphycus* have been transplanted from island to island with most of the material originating

in the Philippines. The main route has been from the Philippines to Hawaii and Kiribati, and from the Philippines to Tonga and Fiji. The Marshall Islands obtained their stock from both the Philippines and Kiribati. Most other countries source stock from Kiribati or Fiji.

The amounts transplanted among islands have ranged from as little as 14 kg to 0.7 t. On only one occasion, a shipment from Fiji to the Solomons in 1988, has there been any documented attempt to quarantine the plants in order to minimise the risk of importing associated species or any diseased plants. There are anecdotal reports of quarantine procedures being followed in Fiji, Vanuatu and Marshall Islands, but we do not know what these procedures were.

Quarantining is important to minimise the risk of accidental introductions, and also to establish if the species being introduced is likely to become a pest itself. So far there have been no reports in the South Pacific of *Kappaphycus* becoming invasive and a pest, but in Hawaii, reports to this effect have been published recently. So we should not presume that *Kappaphycus* will always remain a benign species at new locations. The main quarantine problem, however, is that of preventing accidental introductions of associated species; and when volumes exceeding half a tonne are sometimes transplanted this is a real risk.

SPC has therefore developed a protocol for translocating *Kappaphycus,* and commissioned IMR to field-test their protocols. The protocol is fairly simple and involves washing and cleaning the specimens before they are dispatched and upon arrival, and keeping the plants in quarantine for two weeks during which period there is further cleaning and washing. This protocol is intended to remove most if not all macrobiota, but obviously will not remove the microflora such as diatoms, dinoflagellates and protozoa living on the surface of the seaweed. Nor will it isolate internal parasites such as viruses, fungi or protozoa, although plants that are obviously diseased would be removed.

Accordingly, the IMR obtained shipments of *Kappaphycus* from three Fijian farms to test this protocol. The morphology of *Kappaphycus* is much influenced by environment, especially wave action, and the plants that we received exhibited

very different morphologies. We discovered that compared with many other seaweeds, *Kappaphycus* supports a relatively sparse macrobiota. This is particularly so for laxly branched, long slender plants from Macuata and Savusavu. Compressed, ball-like specimens from sites with relatively high wave action, such as Bua, provided more nooks and crannies for phytal flora and fauna. Hosing, and gently scrubbing the plants with filtered seawater proved to be an effective means of dramatically reducing the epibiota on the specimens. After two weeks, untreated plants had a much greater diversity and abundance of macrobiota than did the washed plants. Some species were however, persistent, especially several types of filamentous epiphytic algae, whose bases are embedded in the *Kappaphycus* tissue. Though these epiphytes can be picked or scrubbed off, they quickly regrow. In the absence of any strong water movement in our culture tanks they looked likely to overgrow the specimens unless the cleaning process was maintained. Although washing removed most macrobiota, handling the plants evidently caused stress, which resulted in the treated plants growing more slowly than those untreated. However all specimens exhibited significant growth over two weeks. Another problem was that after a week, many plants lost colour and became necrotic at their tips. This indicates the problems of a closed tank system where seaweeds are likely to become nutrient limited.

We concluded that the washing and quarantine procedure was effective at removing most large epifauna, but it would not prevent the introduction of some small epiphytes embedded in the host's tissue. Microscopic examination of periodic washings also showed that washing did not significantly reduce the surface microbiota.

Several simple improvements to this procedure could be made. Perhaps the simplest, is to wash the plants in fresh water which would be more effective at removing the animals, and which is possible because *Kappaphycus* tolerates low salinities for short periods. Second we would recommend experimenting with surface disinfectants to try to kill epiphytes and epifauna. Brief immersion in copper sulphate may kill epiphytic algae including phytoplankton and even fungi while Betadine (an iodine based antiseptic) or chlorine would eliminate a wide

range of microorganisms. Experiments are needed to test dosage and exposure periods. If successful, then surface disinfection could reduce the quarantine period.

Another stratagem is to minimise the volumes of the seaweed that are transplanted. One method is to minimize the mortality of the transplants at their new location so that there is an opportunity to “bulk up” the specimens to provide the desired biomass needed to supply cuttings; ie establish a nursery for the plant. Otherwise countries will continue to import very large volumes, anticipating high mortality, when the risk of introducing unwanted species increases with the volume—possibly exponentially. Another method, best adopted, is to transplant only the apical parts of plants because these are relatively free of epiphytes and animals. Where countries want axenic cultures of *Kappaphycus,* then tissue culture is really the only option. This requires considerable expertise and equipment at source and also at the destination where the cultures may have to be maintained for as long as four years before there is sufficient material to provide cuttings for an experimental farm.

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[1] Picture obtained from http://www.seafdec.org.ph/downloads/kappa.pdf on 14th Nov 2003.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ASC	Atoll Seaweed Company Ltd (of Kiribati)
CFTC	Commonwealth Fund for Technical Cooperation.
CI	Conservation International
CMI	College of Marshall Islands
EU	European Union
FAO	Food and Agriculture Organisation of the United Nation
FFA	Forum Fisheries Agency
ICLARM	International Centre for Living Aquatic Resources Management (now changed to World Fish Centre)
IMR	Institute of Marine Resources of The University of the South Pacific
JOCV	Japan Overseas Cooperation Volunteer
MIDB	Marshall Islands Development Bank
MIMRA	Marshall Islands Marine Resources Authority
MSP	Marine Studies Programme of The University of the South Pacific
PDF	Provincial Development Fund (managed by FFA)
PICs	Pacific Island Countries
RFEP	Rural Fisheries Enterprises Project (A Solomon Islands fisheries project funded by the European Union)
RMI –	Republic of Marshall Islands
SEAFDEC	South East Asia Fisheries Development Centre
SIDT	Solomon Islands Development Trust
SPADP	South Pacific Aquaculture Development Programme
SPC	Secretariat of the Pacific Community

SSP	Seaweed (South Pacific) Ltd
USA	United States of America
USP	The University of the South Pacific

GENERAL INTRODUCTION

Strategically, quarantine agencies try to prevent or minimize the risk of introducing unwanted species—especially pests and diseases. Tactically they try to enforce a comprehensive border control by refusing entry to all potentially harmful species or products including non indigenous species, foreign food products and people with communicable diseases. Historically quarantine has largely focused on preventing the introductions of diseases and pests harmful to humans and to agriculture and forestry.

There is increasing concern about the ease by which a wide range of plants and animals is translocated around the world as the technology, speed and the volume of transport systems improves and increases. Introductions via ships' ballast water is a topical example. Quarantine agencies are overwhelmed; often being reduced to randomly inspecting as little as 5% of shipping containers for example. Most governments have little ability to impose any truly effective quarantine on ships' ballast water. These new introductions are breaking down biogeographic barriers that permitted allopatric speciation and endemism and which isolated very different regional biotas for eons. The breakdown is homogenizing the planet's biota (Mooney and Hobbs 2000), and it is causing the decline of many indigenous species. Because when non-native and native species have similar living requirements, the native species are usually out-competed by the invaders.

Because all countries intentionally import various live organisms for agriculture and commerce, quarantine agencies, in addition to border control, are required to minimize the risk of such intentional introductions. They must ensure that such species are unlikely to become pests once they are released and that no other associated species are accidentally introduced with them.

This is a difficult and demanding task, because usually it is impossible to predict how a newly introduced species will interact with an already established biota

comprising non-native and native species. Particular attention must be paid to what the new immigrant is likely to eat, how rapidly it grows and breeds relative to native species and how rapidly it is likely to be dispersed. But despite such precautions there is always a significant ecological risk in importing any new species however commercially desirable the introduction appears at the time. Previously and even today, the decision to import a species is often based mainly on an assumption that likely commercial benefits outweigh unknown ecological costs—the rationale used by FAO to justify the introductions of several freshwater fish species to Papua New Guinea in the 1990s. But increasing numbers of case histories where the problems caused by intentional introductions have far exceeded benefits, are causing many governments to take a more precautionary approach. Thus requests to introduce Californian red-foot abalone,Tasmanian freshwater crayfish and several other freshwater species to New Zealand have been refused by the New Zealand Government in recent years.

Of course not all new introductions are harmful or devastating. In many cases they have made enormous social and economic contributions to the benefit and welfare of humanity. Here in the Pacific, a non-marine example is the introduction of the sweet potato, originally from South America but more recently (1800's) from New Zealand to several South Pacific Countries. Historically Pacific islanders transplanted staples such as cassava, breadfruit and taro, useful species like bamboo, as well as pigs and chickens. And in recent years a seaweed introduction—that of *Kappaphycus alvarezeii*—has become an important cash crop to land-strapped nations such as Kiribati.

An easier task for quarantine agencies is to minimize the risk of accidentally introducing other species associated with the intended introduction—which is why quarantine agencies confine animals for several months, years and even for several generations, at isolated locations to try to identify if they are carrying any serious infectious diseases.

Mariculture and aquaculture are rapidly developing industries. About a fifth of all fish landings in the world comprise species that have been farmed and reared in ponds, raceways, sea cages and the like (Coull 1993). Compared with the wild fisheries, aquaculture is based on relatively few species that are often traded and translocated. There is always a risk that some of these species may escape from farms and become pests in their new environments, or that associate species may be accidentally introduced. In Fiji the intentional introduction of Asian carp resulted in accidentally introducing larvae of the Asian water snail, *Viviparus japonicus*, now spreading into the waterways of Fiji (Haynes 2001). Numerous species were translocated around the world with shipments of the Pacific oyster (*Crassostrea gigas*). For example, in the 1980s, in l'Etang de Thau. on the French Mediterranean coast, at least three large Japanese seaweeds (*Laminaria japonica*, *Sargassum muticum* and *Undaria pinnatifida*) were accidentally introduced, presumably as spores or gametophytes, with spat of the Pacific oyster sourced from Japan and Korea (Meinesz 1997, p26). The *Undaria* kelp has now spread or been spread around the French Atlantic coast (Pérez *et al* 1984) and to the south coast of England.

Some seaweeds are of commercial importance. For example, *Undaria* pinnatifida, mentioned above, is extensively farmed as a sea-vegetable in Korea Japan, China who since 1990 have collectively produced between four and eight thousand tonnes of wet weed per year. The most important seaweed industry by far is the mariculture of the red seaweed *Porphyra yesoensis* which is used to make various types of *nori* (Nisizawa *et al.* 1987). Though this industry is largely based in Japan and China, the seaweed has been exported to the Pacific and Atlantic coasts of North America with little regard as to what the ecological consequences of this introduction might be.

Many seaweeds have been accidentally translocated around the world. Eldredge (1994) quoting Russell (*pers comm*) reported that some 150 species of marine algae (seaweed), including species found in ballast water, have been

translocated; at least a half by ships, and about a half with aquaculture experiments, and some via canals such as Suez.

Of these, relatively few have become serious ecological pests, although several have become very widely distributed and abundant in their new habitats—a species of the red seaweed *Asparagopsis* introduced to the North Sea probably from Australia (Elton 1958), and the introduction of Asian *Undaria pinnatifida* to France, New Zealand, Australia and Argentina (eg Hay 1990) are well known examples. At least three species are considered to be serious pests: Asian *Sargassum muticum* introduced to the Pacific seaboard of North America and to northern Europe, the introduction of a variety of *Caulerpa taxifolia*, possibly from northern Australia, to the Mediterranean (Meinesz 1997) and most recently to southern California, and the introduction of *Codium fragile* subsp *tomentosoidies* from Europe to the Atlantic seaboard of North America (Carlton and Scanlon 1985) and to Australia [http://www.epa.vic.gov.au, Publication 67, April 1999].

Although not macroscopic seaweeds, several microscopic algae such as dinoflagellates have become pests in certain regions. Of concern to the Tasmanian and New Zealand mariculture industries has been the appearance of a toxic species, *Gymnodinium catenatum*, in Hobart Harbour, which closed shellfish harvesting in Tasmania for six months in 1986 and 1991 (Hallegraeff and Bolch 1992). Its presence in Hobart caused the New Zealand Government to prohibit ballast water discharges from ships arriving in New Zealand from Tasmania. The appearance of this species has been attributed to discharging ballast water probably uplifted in the northwest Pacific. Dinoflagellates form resistant cysts that sink into ballast tank sediments and are thus readily transported around the globe in ships' ballast (Hallegraeff *et al.* 1990, Hallegraeff 1992). The dinoflagellate, *Alexandrium catenella*, which causes paralytic shellfish poisoning, and which has been appearing seasonally off the northeast coast of North island, New Zealand may also be a non-indigenous species introduced via ships' ballast water.

In New Zealand, mariculture of the green-shell mussel, *Perna canaliculus*, worth more than $NZ100 million per year, is largely dependant on shipping beach-cast seaweed, covered with mussel spat, from beaches in northern New Zealand to the Marlborough Sounds in the South Island. Of concern is that toxic or potentially toxic species of phytoplankton found in northern New Zealand may accidentally be introduced to the Marlborough Sounds via the seaweed to which they are attached. As a result there, internal quarantine procedure has been established. This requires washing samples of all seaweed shipments and microscopically examining the washings to check for the presence of toxic phytoplankton before permission is granted to air-freight the seaweed/spat samples to the Marlborough Sounds.

Here in the tropical and subtropical Pacific several seaweeds are eaten as a vegetable by the indigenous people but these are mainly collected in the wild. The only seaweed cultivated to any extent is the red, carrageen, seaweed *Kappaphycus alvarezii* which is grown on ropes and sticks in lagoons in several countries. The seaweed is air-dried and shipped by the container load to factories in North America and Europe where carrageen is extracted. On many islands the cultivation of this seaweed has become a means of making a small cash income with minimal capital investment.

The native range of *Kappaphycus* (and of a similar genus *Eucheuma*) was probably Southeast Asia. Today, however the plant is widely distributed throughout the Pacific as a result of various entrepreneurs, governments and regional agencies transplanting fragments of the plant to new locations. Nowhere does *Kappaphycus* appear to have become an ecological problem or a serious pest although the seaweed has spread from its original farms. In the Solomon Islands, for example, attempts to farm *Kappaphycus* at two locations which were later abandoned resulted in the plant dispersing to and persisting at adjacent sites.

This does not mean, however, that *Kappaphycus* will be ecologically benign in all new locations. Also, because large quantities of the seaweed are usually transplanted, eg up to 0.5 tonnes at a time, there is always the danger of accidentally introducing associate species, eg crustaceans, or *Kappaphycus* specimens that are infected with viral, fungal or other diseases which are already problem for some *Kappaphycus* seaweeds, or which may become a problem for farmers growing species like shrimp. Also, as the New Zealand example mentioned above shows, there is possibly a danger of translocating harmful dinoflagellate species with shipments of *Kappaphycus*. Certain parts of the Pacific are much more ciquatoxic than others, and there is a danger that the toxic dinoflagellates, or their cysts, which cause fish poisoning may be attached to specimens of *Kappaphycus* destined for a new location. Hence the need to quarantine this seaweed before it is released.

Purpose of this report

This report is mainly concerned with validating Secretariat of the Pacific Community (SPC) protocols for translocating *Kappaphycus alvarezii* among Pacific island nations. In this regard The IMR was commissioned by SPC to:

(1) conduct a literature review on the history of introductions of *Kappaphycus alvarezii* in the South Pacific region and the current status of *Kappaphycus* mariculture in these countries and

(2) to field test quarantine protocols for the introduction of *K. alvarezii* to other Pacific Islands Countries (where they do not occur naturally) for mariculture.

This report presents the results of the above exercises in two “stand alone” sections. **Part A** discusses and describes the history of *K. alvarezii* introductions and the current status of seaweed mariculture in the Pacific. **Part B** discusses quarantine issues and presents the results of field-test on the proposed SPC quarantine protocols.

PART A: INTRODUCTIONS OF KAPPAPHYCUS ALVAREZII IN PACIFIC ISLAND COUNTRIES

Introduction

The earliest publication reporting on the economic potential for seaweed colloids was by Solly and Booth (1977). Some reasons being stated as to why *Kappaphycus* is potentially an ideal industry for the Pacific Island Countries (PICs) is that it requires low capital investment, low technology, no refrigeration, is environmentally friendly and is normally compatible with traditional fishing and other subsistence uses of inshore marine resources. Hence it can be easily integrated into the subsistence life style of most PICs (Solly and Booth 1977, South 1993). The diversified market for seaweed products in areas of food, pharmaceuticals and other associated industries would always ensure a demand for seaweed (SEAFDEC 1988). Furthermore it can be integrated with fish or prawn farms to absorb excess nutrients from the farms. Seaweed as an industry can be an alternative to other rural industries such as copra and fishing for finfish, molluscs, bêche-de-mer and others. This would be a welcome diversification to the economic base of the South Pacific countries.

Following the great success in marine agronomy of carrageenophytes as a village level industry in Philippines, attempts to establish a seaweed industry in the Pacific were made in the periods between 1970's to late 1980's. Specimens of *Kappaphycus alvarezii* (Doty) Doty (formerly known as different species of *Eucheuma*) were brought from the Philippines as seed materials (South 1992). Seaweed farming as an industry has undergone varying developments since it started in the Pacific. In some locations for example, The Solomon Islands, it never progressed beyond the trial stages when it was first introduced and second trails are currently undergoing, while in Kiribati it has become an important industry contributing to national economy and development.

Historically carageenophytes in the Pacific have been described as species of *Eucheuma* and *Kappaphycus. Eucheuma* species produced *iota* carageenan and *Kappaphycus* species produce *kappa* carageenan. This report considers only the *Kappaphycus* species (var. tambalang and sacol). According to South (1993), Luxton (2003), Ask *et al*. (2003a) or Ask *et al.* (2003b) the two varieties *of K. alvarezii* were introduced to several Pacific Island Countries. The terms "*Kappaphycus*" and "seaweed" will be used interchangeably in this report.

A history of introductions of *Kappaphycus* to the Pacific has been described by several authors and it has also been the subject of technical papers by some Fisheries Departments of the region. Examples include Solly and Booth (1977), Why (1985), Luxton *et al.* (1987), Nelson (1988) Adams and Foscarini (eds) (1990), Smith (1991), Ram (1991), McLachlan (1992), South (1993) Eldredge (1994), Luxton and Luxton (1999), Ask *et al*. (2003a) and Ask *et al.* (2003b). Reviewed below is the history of introductions to the Pacific Island countries and the current status of seaweed farming in the respective countries. In a lot of cases it has not been possible to obtain accurate figures and it has to be inferred from literature and reports.

Cook Islands

There is no commercial seaweed farming in the Cook Islands at present. *Kappaphycus alvarezii* was introduced to the Islands of Aitutaki in the late 1980's from Fiji (Eldredge, 1994), but it never progressed to become a successful mariculture activity. A second attempt at re-kindling interest was made in 2001 when 700kg of seaweed was brought from Tabuaeran Lagoon in Kiribati destined for Tongareva, Rakahanga and Pukapuka. The Introduction to Tongareva Islands was refused by the Island Council at the last hour due to fears of inadvertently introducing unwanted foreign organisms with the seaweed, which might jeopardise the local pearl oyster industry. Introduction were, however, made to Rakahanga and Pukapuka, although Luxton (2001) had reported that achieving viable commercial plots was not possible in those locations. Other suitable sites

with expressed interest from residents, which were not tried, were Palmerston, Tongareva and Aitutaki lagoon. Luxton (2001) recommended maintaining seed stock in Rakahanga for trials in other locations at a later date. It is not known whether this recommendation was implemented. From a commercial viewpoint this second attempt at introducing *Kappaphycus* to the Cook Islands in 2001 was unsuccessful.

Federated Staes of Mirconesia

Eldrege (1994) reported that *K. alvarezii* was observed to Pohnpei and Kosrae for experimental culture. The exact time of when this introduction was made was not recorded and no further details provided, except that mariculture of *K. alvarezii* never progressed to commercial scale.

Fiji

Kappaphycus alvarezii was first introduced to Fiji from the Philippines in February 1976 (Solly and Booth 1977). Solly and Booth (1977) reported that four growing sites were established, three in Southeast Viti Levu and one off the coast of western Viti Levu. Good growth rates were reported initially, but later on growth rates declined as a result of ice-ice disease and a cyclone. Eventually the entire crop was lost in August 1976 (Luxton *et al*. 1987)

A second introduction to Fiji was made in April 1984 from Tonga with seed material originally obtained from the Philippines (Luxton *et al.* 1987). Propagules were planted at four sites on the barrier reef north of Rakiraki using monoline techniques[2]. Propagules from these plots were used in trials to establish a pilot farm. Four cyclones disrupted the trials, however, but despite the difficulties, the mariculture expanded to other parts of Fiji (Tavua, Moturiki and Kaba) (Luxton *et al.* 1987). Collaborative efforts between the Industrial Development Unit of The Commonwealth Fund for Technical Co-operation, the Fijian Government and the phycocolloid industry ensured a steady increase in farming effort.

[2] Fragments of seaweed are attached to a single rope about 30 m long and pulled taut between poles.

Considerable expansion in 1987 resulted in 240 farms producing 217 t for export (South 1993). Coast Biologicals Limited, a New Zealand company were responsible for marketing the *Kappaphycus*. The 1987 coup in Fiji resulting in political instability, coupled with the effects of cyclone Bola were major setbacks to the seaweed development in the late 1980's resulting in Coast Biologicals Ltd withdrawal from Fiji in 1988 (South 1993). A strengthening of New Zealand dollar against the U.S dollar was also a contributing factor as were trade sanctions on Fiji (Robertson 1990), and the farmers diversion to the short lived beche-de-mer "boom" fishery (Prakash 1990). With the withdrawal of Coast Biologicals Ltd, the Fisheries Division took over the development of seaweed as an industry concentrating on mainland Viti Levu (Malake, Tavua and Kiuva). The National Marketing Authority (NMA) was responsible for marketing the dried seaweed. The Fiji Government during this period (late 1980's) was managing the industry pending interest from potential investors (Mario *pers commm*.). Annual Exports of dried *Kappaphycus* between 1984-1990 are given below (Table 1)

Table 1: Annual exports of dried *Kappaphycus* in Fiji, 1984-1990.

Year	**Export (t)**	**No of Farms**
1984	Growth trials	-
1985	30	35
1986	200	160
1987	217	240
1988	60.5	30
1988	60.3	-
1989	80.3	-
1990	87.4	-

(Adapted from Prakash 1990 & South 1993)

A new company, Seaweed (South Pacific) Limited (SSP), was formed in 1989 with New Zealand, Fijian and Australian capital. It established a private farm near Savusavu on Vanua Levu (Prakash 1990). SSP had hopes of being fully operational by mid 1990. They anticipated producing 300 t in the first year, 600 t in the second year and 800 t in the third year. SSP had plans to take over the

Kappaphycus marketing in Fiji and possibly the Pacific islands with a view to constructing and operating a processing plant (SSP Ltd. Proposal 1988). The aspirations of SSP however came to an end when heavy swells following a cyclone washed off a significant amount of seaweed from the farm lines. SSP did not have sufficient capital to finance re-planting, this resulted in its closure in 1991 (Mario *Pers. comm*).

After the collapse of SSP, the Fiji Fisheries Department took over the seaweed project for about six months (Jan-June 1991). In that half year period only 20 t of dried *Kappaphycus* was exported (Mario *Pers. Comm.).* Oceania Trading Ltd. then took over the marketing role from 1991 to 1993 and during this period, the total export was just 30 t. The effects of Cyclone Kina in 1993 resulted in the complete closure of the industry until 1997. The market, created with FMC corporation was lost, and there were no funds to continue developing the seaweed industry because the government had to concentrate efforts and resources on post-cyclone rehabilitation (Mario *pers comm*).

Seaweed farming was restarted in 1997 when funds were made available by the government through the Commodity Development Framework (CDF). Seed stock was obtained from locations where farms used to operate (Fulaga, and Onea in Lau). The Fiji Fisheries Department purchased dried seaweed from the growers at fortnightly intervals until 2001 when a new Fijian company, REL Fisheries took over the marketing. Locations of farms around Fiji and the number of farmers since 1997 are given below (Table 2).

Table 2: Location of seaweed farms around Fiji and a comparison of the number of farmers involved in farming seaweed in 1997 and 2003.

Province	**Farming locations**	**Number of farmers in 1997**	**Number of farmers in 2003**
Lau	Fulaga and Onea	14	0
Lau	Ono	97	20
Lau	Namukailau	31	0
Tailevu	Kiuva	14	2
Ba	Yasawa	22	9
Cakaudrove	Nakobo,Karoko and Tawake.	157	Less than 20
Macuata	Namuka, Kaveoa, Tilagica, Druadrua	64	10
Serua	Serua, Vunaniu	40	0
Lomaiviti	Moturiki, Daku, Uluibau, Nukutolia	42	0

(Mario *pers comm*)

As shown in Table 2, the number of farmers has declined from 481 in 1997 to 41 in 2003 – a decline of 93 %. This has been attributed in part to disease outbreaks in 2001, epiphytic filamentous algae, ice-ice disease and diebacks. The disease problems were considered to be minor difficulties which could be easily overcome (Mario *pers comm* 2003). The major problem causing the decline was poor marketing arrangements within Fiji, despite the fact that an export market for Fiji (FMC Corporation) is assured. Farmers currently complain of considerable delays in selling of their seaweed especially in some cases where the exclusive FMC marketing agent (REL Fisheries) do not buy seaweed for up to six months. This is a very long purchasing time compared to the fortnightly purchase previously made by the Fisheries Department. Farmers also complained of not receiving payments from the marketing agent and subsequently shipping masters refused to transport seaweed within the Fiji group due to non payment of over due freight bills incurred by REL Fisheries. The current problems in marketing do not bode well for seaweed farming and if they are not solved it may cause the seaweed industry in Fiji to collapse again. Marketing problems are also reflected in the declining trend of seaweed exports between 1997-2003 as shown in Table 3.

Table 3: Seaweed exports from Fiji, 1997-2003.

Year	**Export (t)**
1997	Development
1998	19.8
1999	300
2000	515
2001 (REL Fisheries took over marketing)	240
2002	80
2003	Farmers reported no purchase of seaweed by the company since the beginning of the year.

Mario (*pers comm*) offers the view that the privatising of the marketing component of seaweed from the government to the private sector in Fiji was premature. At such an early stage it needed the Fisheries Department and the National Marketing Authority for further development and technical support. Citing the example of Kiribati, Mario (*pers comm*) argued that it took 11 years for the industry to develop as a fully-fledged industry. Farmers require encouragement and incentive to produce consistently large volumes of around 1700 t per year for the industry to become economically viable. Once such productivity is achieved then that is the time for the private industry to become involved.

French Polynesia

Eldredge (1994) documented the introduction of *K. alvarezii* to French Polynesia around the 1980's. No details were provided and initial developments were reported as being successful.

Kiribati

The seaweed *Kappaphycus alvarezii* and *Eucheuma denticulatum* from Hawaii were first introduced to Kiritimati (Christmas) Island by Dr. Maxwell Doty (Why 1985, Uan 1990, South 1993, Luxton and Luxton 1999) and to Tabuaeran (Fanning Is.) (also from the Hawaiian seedstock) by Russell in 1977 (Luxton and Luxton 1999). Growth studies to determine farming potential were conducted by the Kiribati Ministry of Natural resources in 1980 on Kiritimati Island. This pilot project was terminated in 1981 when wave action was too strong causing a 75% reduction in productivity. The seedstock was therefore transferred to the Tarawa lagoon in the main Kiribati chain (Why 1981). A trial shipment of two tonnes was made to USA in 1981 as an attempt to gain interest. USA buyers showed little interest preferring to grow and process their own crops (Why 1985).

In 1982, trials were established in the sheltered Marakei Island lagoon to compare growth rates with those obtained at the exposed Kiritimati Island site. However growth was reported to be slow due to poor tidal circulation and the long term residence times of water entering the lagoon, resulting in reduced nutrient circulation. Transplanting to smaller outer islands of Kiribati (eg. Onotoa) was made between February and June 1983 (Why 1985). Growth rates of 1.8-4.5% day^{-1} varied with location. Important growth limiting factors included the effects of epiphytes, grazing by herbivorous fishes, occurrence of ice-ice disease and the effects of westerly winds (South 1993). Six Kiribati islands that were identified as favourable sites for seaweed farming were; Tarawa, Butaritari, Aranuka, Abemama, Abaiang, and Beru (Uan 1990).

These trials resulted in commercial production by 1985 (Luxton and Luxton 1999). Uan (1990) reported that there was a total of 2,107 farmers involved in seaweed farming between 1985 to 1990. The industry however declined in 1990, and Luxton and Luxton (1999) attributed this to lack of business infrastructure, poor crop quality and few export markets.

The Kiribati Government intervened and restructured the industry in 1992, when a state owned corporation, Atoll Seaweed Company (ASC), was formed. This new company secured a five year forward supply agreement with the company, Copenhagen Pectin A/S in Denmark (Luxton and Luxton 1999). ASC was responsible for reintroducing of *K. alvarezii* to the Line Islands (particularly Tubuaeran and Kiritimati) where seaweed farming proved successful. The annual dried seaweed production in Kiribati since the commencement of seaweed farming in 1985 to 2002 is given in the table below. These data were supplied by the Atoll seaweed Company of Kiribati. According to the ASC (pers. Comm. 2003), the recent tourism boom from visiting cruise liners in the main seaweed producing Islands of Kiritimati and Fanning Island has affected seaweed production in these areas. A lot of people have deserted seaweed farming for the more lucrative tourism business.

Table 4: KIRIBATI SEAWEED PRODUCTION BY ISLAND, 1985-2002 (MT)

ISLAND	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Butaritari	0.9	13.0	5.0	0.1	0.2		0.3		1.0						3.7	1.6	2.2955	0.436
Marakei							0.4					23.0	31.0		7.0	11.7	0.1325	0
Abaiang		2.0	5.0	20.0	82.0	581.0	684.0	290.0	157.0	242.0	32.0	38.0	5.7	0.5	0.3	4.8	0.1614	0.046
N Tarawa							1.0				0.4	1.0			0.6	0.1		0
S Tarawa	24.0	45.0	12.0	6.0	15.0	2.0	2.0				1.0	2.0			0.6	1.3		0
Maiana							3.0	15.0	19.0	82.0	211.0	162.0	111.0	1.2	7.8	0.4	3.1	0
Aranuka		3.0	3.0	4.0	2.0						0.5	8.0		3.1	13.5	13.3	0.025	0
Abemama		3.0	5.0	15.0	50.0	54.0	189.0	38.0	4.0	9.0	48.0	83.0	24.0	4.8	11.4	22.5	5.6355	0.933
Nonouti													0.2					0
Tab. North							11.0	11.0	2.0	7.0	25.0	126.0	38.0	0.9	0.3	0.2	0.806	0
Tab. South												0.2	0.1				0	0
Beru														2.3	2.4			0
Onotoa							129.0	80.0	22.0				0.4					1.3579
Gilbert Islands	24.9	66.0	30.0	45.1	149.2	637.0	1019.7	434.0	205.0	340.0	317.9	443.2	210.4	12.8	47.4	56.0	12.2	2.7729
Kiritimati										24.0	125.0	309.	140.0	11.4	50.3	49.6	18.7755	2.461
Fanning										32.0	212.0	494.0	574.0	718.0	1072.5	1332.0	1129.2976	525.5585
Line Islands										56.0	337.0	803.0	714.0	729.4	1122.8	1381.6	1148.1	528.0195
Total	24.9	66.0	30.0	45.10	149.2	637.0	1019.7	434.0	205.0	396.0	654.9	1246.2	924.4	742.2	1170.3	1437.5	1160..2	530.7924
	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002								
Gilbert Is	205.0	340.0	317.9	443.2	210.4	12.8	47.4	56.0	12.2	2.8								
Line Island	0.0	56.0	337.0	803.0	714.0	729.4	1122.8	1381.6	1148.1	528.0								
Total	205.0	396.0	654.9	1246.2	924.4	742.2	1170.3	1437.5	1160.3	530.8								

Table 5: 2003 MONTHLY KIRIBATI SEAWEED PRODUCTION BY ISLAND (MT)

ISLAND	Totals	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Butaritari	0.252	0.000	0.142	0.110									
Bikati (Buta Islet)	0	0.000	0.000	0.000									
Marakei	0	0.000	0.000	0.000									
Abaiang	0.0554	0.055	0.000	0.000									
Nuotaea (Abaiang Islet)	0	0.000	0.000	0.000									
South TRW	0	0.000	0.000	0.000									
North TRW	0	0.000	0.000	0.000									
Maiana	0	0.000	0.000	0.000									
Abemama	5.4691	0.115	0.044	0.046			1.047	2.151	2.066				
Abatiku (Abema Islet)	0	0.000	0.000	0.000									
Aranuka	0	0.000	0.000	0.000									
Takaeang (Aranuka Islet)													
TabNorth	0	0.000	0.000	0.000									
TabSouth	0	0.000		0.000									
Onotoa	3.7034	0.4764	1.303	0.178	0.3418	0.09	0.358	0.4606	0.4956				
Nonouti	0	0.000	0.000	0.000									
Beru	0	0.000	0.000	0.000									
Total Gilbert	9.4799	0.647	1.489	0.334	0.342	0.090	1.405	2.612	2.562	0.000	0.000	0.000	0.000
Cumulative		0.647	2.136	2.470	2.812	2.902	4.307	6.918	9.480	9.480	9.480	9.480	9.480
Kiritimati	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fanning Baerau	133.362	28.037	27.762	24.408	16.618	23.792	12.745	0.000	0.000	0.000	0.000	0.000	0.000
Fanning Tereitaki	45.7765	7.903	12.904	6.436	9.667	8.868	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fanning Total	179.1385	35.940	40.666	30.844	26.285	32.660	12.745	0.000	0.000	0.000	0.000	0.000	0.000
Total Line Group	179.1385	35.940	40.666	30.844	26.285	32.660	12.745	0.000	0.000	0.000	0.000	0.000	0.000
Cumulative		35.940	76.606	107.449	133.734	166.394	179.139	179.139	179.139	179.139	179.139	179.139	179.139
Grand Total	188.618	36.587	42.155	31.178	26.626	32.750	14.150	2.612	2.562	0.000	0.000	0.000	0.000
Cumulative		36.587	78.741	109.919	136.545	136.545	169.295	183.445	186.057	188.618	188.618	188.618	188.618

Republic of Marshall Islands (RMI)

Two species of red algae *E. denticulatum* and *K. alvarezii* were introduced into Mil and Likiep in Majuro lagoon in 1990 (Eldredge, 1994). There is no other information available relating to those early trials. In 2002, Marshall Islands began a new seaweed farming project with seedlings imported from Kiribati under the expertise of Mr James Uan from Kiribati. The Food and Agriculture Organisation (FAO), Marshall Islands Marine Resources Authority (MIMRA), Marshall Islands Development Bank (MIDB) and the College of Marshall Islands (CMI) supported this initiative. Given the similarity in geography and environment, RMI aims to closely follow Kiribati as a case study for their seaweed farming activities. It should be noted however that socio-economically, these two countries are very different since RMI benefits from a pact of association with USA.

A suitable site was identified in Majuro lagoon and with the assistance of FAO. A pilot farm is well under way and being closely monitored by MIMRA with assistance from students of CMI. It is anticipated that this pilot farm should provide enough seedlings for further grow-out for other farms in the RMI. The project also aims to involve outer island communities as well as to provide an extra source of income, job and social security. The success or failure of this pilot project will decide future sites, farming methods and seaweed farming in the RMI. The project is still ongoing and will end in Feb 2004. (Glenn Joseph, *pers comm* 2003)

Solomon Islands

Specimens of *K.alvarezii* were introduced into the Solomon Islands in February 1988 from Fiji (Smith 1991). Smith (1991) reported that the Fiji Ministry of Primary Industries issued a phytosanitary certificate for the seaweed. On arrival in the Solomon Islands the seaweed were maintained in quarantine raceways at the ICLARM (now known as WorldFish Centre) facilities in Aruligo for 14 days. The purpose of the quarantine was to clean the weeds of any `hitch hiking'

invertebrates rather than to prevent the spread of any infectious diseases. During the quarantine period, the seaweed increased in weight from 14 Kg to 17 Kg. However they also suffered from ice-ice and changed to a more elongated morphology. Due to the problems of ice-ice and in the absence of any record of infectious diseases from literature, Smith (1991) reported that subsequent shipments from Fiji were kept for periods shorter than 14 days.

Mariculture trials were funded by a British Aid project from 1988 to 1991 (Smith 1991). Locations where trials were conducted were, Rarumana and Vona Vona in the Western Province, North Malaita and in Ontong Java. Good growth rates were achieved. Smith (1991) reported that daily growth rates in the Western Province ranged between 1 - 4.9% day^{-1} while in Ontong Java it ranged from 1.5-3.2 % day^{-1}. No growth rates were recorded for North Malaita.

Seaweed farming was developed to semi-commercial levels with around 3 tonnes produced, however bad weather, grazing by herbivorous fishes and other social commitments by farmers resulted in a decline in production (Smith 1991). A trial export failed when the company Seaweeds (South Pacific) Ltd, based in Suva, Fiji went bankrupt and wound up operations. The failure of the Industry in Fiji together with the lack of interest from buyers and the inability to interest farmers to produce large volumes of seaweed was also a major factor resulting in the failure of the industry to make any progress beyond the trial stage. There was no seaweed farming in Solomon Islands from 1991-2000.

In early 2001, the Rural Fisheries Enterprises Project (RFEP) supported by funding from the European Union made attempts to revive and develop seaweed farming. A seed-stock farm was planted at Rarumana from the `1991 abandoned stock' which had taken hold and had spread to different parts of Vona Vona and Roviana lagoon. Farms were developed in Rarumana and Vona Vona Lagoon. The main culture method employed is the "off bottom monoline" method. In 2002, the Solomon Islands Development Trust (SIDT) assisted farmers in Langa Langa

lagoon in Malaita to develop seaweed farming, employing both the monoline and raft method of farming. In 2003 seaweed farming has developed in Wagina and Taro island in Choiseul, Kia and Buala in Isabel province, Lau Lagoon in North Malaita, Maramasike area in Are Are, Malaita and in some parts of Reef Islands in Temotu Province. Expansion to Marovo Lagoon is expected to occur before the end of 2003 (Alex Meloty *pers comm*).

The current (2003) buying price from farmers is SBD$2.00 per kg (equivalent of about FJD$0.53). Seaweed farmers in Rarumana have produced about 18 tonnes of dried seaweed which are currently being stored in what was previously a copra shed. A first shipment of about 35 tonnes will be exported by the end of 2003 (Alex *pers comm*). The decline in copra price will continue to be a motivation for seaweed farming, current buying price for copra is SBD$1.00 per kg. Most farmers in Langa Langa however have refused to sell their seaweed to the RFEP. This was due to what they considered to be a very low price compared to what some politicians have promised them, a price of USD$5.00 per kg. They continue to farm and dry their seaweed and are still waiting for 'the better buyer'. Currently there is significant interest in seaweed farming as an income generating activity in Solomon Islands, and based on the current trend, it will continue to expand to other locations in Solomon Islands in the next few years.

Samoa

The first introduction of two species of *Kappaphycus* (*K. alvarezii* and *K. denticulatum)* into Western Samoa was made in 1974; however there are no details available for the trials conducted with those seaweed (Bell and Mulipola, 1998). The first documented seaweed culture trials was carried out in 1991 at Aleipata and Mulinuu lagoons using *K. alvarezii,* imported from Fiji. The South Pacific Aquaculture Development Programme (SPADP) provided funding assistance. Most of the cultured seaweed was later destroyed by Cyclone Val. About five percent of the seaweed were later recovered and cultured to re-start the seaweed initiative. In 1992 another batch of *K. alvarezii* was imported from Fiji, and together with the earlier seeds, were used to establish a farm in Namu'a. The seaweed grew very well and was ready for harvesting in 5-6 weeks, however, due to other work commitments, the fisheries staff were unable to harvest the crop until 8 weeks later by which time 98% of the crop were grazed by schools of rabbit fish. The remaining 2 percent were transferred to cages for a field grow-out but were later lost to Cyclone Lin. This resulted in the project being discontinued. In 1999 some trials were conducted in Asau Savaii, however the crop was affected by a black worm and since then Samoa has not carried out any seaweed farming trials (Lui Bell, pers. comm. 2003).

Tonga

The first seaweed trials were conducted at Vava'u in 1981 with funding assistance from the Commonwealth Fund for Technical Cooperation (CFTC). Seed stock was brought in from Kiribati and by 1984 six farms were operational on a commercial scale. The success in farming and cooperation between the government and private sector saw the establishment of 36 farms by 1985. Reportedly due to marketing and grazing problems however, the number of farms were reduced by half the following year. Since then Tonga has been struggling to revive their *Kappaphycus* farming industry. In 2002-2003, Tonga has made fresh attempts to revive seaweed farming.

Tuvalu

The first two trials of seaweed farming were conducted in Tuvalu in 1980 with seaweed from Kiribati, this trial however failed. Subsequent trials were conducted in 1990's (again with seaweed from Kiribati) and the latest trial is in 2003 with seaweed from Fiji. All of these trials were conducted on the Island of Funafuti and Nui. A major factor contributing to the failure of the trials was a very high incidence of herbivorous fishes and turtles which graze on the trial plots. Regardless of the previous failure, the Tuvalu Fisheries Department is considering reviewing the programme and making further introductions and trials in the near future (Poulasi, *pers comm* 2003).

Vanuatu

Specimen of *Kappaphycus alvarezii* was introduced into Vanuatu from Fiji in 1999. This project was funded by the FAO South Pacific Aquaculture Development Programme (SPADP). Trials were conducted on Efate (Erakor, Eratap, Lelepa and Paunangisu reefs), Santo (Palekula Bay) and Malekula (Maskelynes and Uripiv Lagoons). The first stock was lost in 2000 due to ice-ice, grazing and cyclone damage. A second stock was imported again in 2001. Most of the second stock suffered the same fate the first stock suffered. A very small stock is currently (in 2003) being maintained at Uripiv lagoon in Malekula, which is sustained by a Japanese Overseas Cooperation Volunteer (JOCV) with funds from the Forum Fisheries Agency (FFA) under the Provincial Development Fund (PDF) for Vanuatu. Pakoa (*pers comm*) is of the view that seaweed farming may not be suitable for Vanuatu because of limited shallow reef areas and regular cyclones. (Pakoa *pers comm*, 2003).

PART B: QUARANTINE PROCEDURES FOR THE INTRODUCTION OF *KAPPAPHYCUS ALVAREZII* TO NEW LOCATIONS

Introduction

The need for quarantining commercially important seaweeds before their release to new locations is discussed in the General Introduction above. To reiterate briefly, there are concerns that large quantities of seaweed being shipped to new locations may contain a phytal fauna such as copepods, amphipods, isopods or polychaete worms, smaller seaweeds attached as epiphytes, a microscopic epibiota possibly including harmful dinoflagellates, and disease organisms within the seaweed tissues. This report will not deliberate further on the subject of quarantine, its rationale or guidelines for Pacific Island Countries. That has already been addressed by earlier SPC publications, eg Humphry (1995).

One of the seaweed species, which has been intentionally introduced to different parts of the Pacific (and to different parts of the worlds), is the red seaweed *Kappaphycus alvarezii* (Solieriaceae, Gigartinales, Rhodophyta). Detailed taxonomic descriptions of *K. alvarezii*, its favourable habitat and subsequent revisions in the nomenclature are cited in Doty (1985) and Doty (1988).

Life cycle of *Kappaphycus* and related species like *Eucheuma* is not well known. Researchers[3] have proposed that it employs a triphasic life cycle with gametophyte (N), tetrasporophyte (2N) and carposporophyte (2N) phases. Details of such a life cycle can be cited in commonly available phycology textbooks (eg South and Whittick, 1987: 142). Sparse knowledge known so far about the male sexual thalli indicate the employment of different life histories[4] (depending on seasons and environmental conditions) with deviations from the ideal triphasic life cycle. Paula *et al.* (1999) and Oliveria and Paula (2003) reported that putative sterile clones, which were initially propagated using tissue

[3] Cited in http://www.surialink.com/abc_euchema/3/18.htm on 7th Oct 2003

[4] A life history is a theoretical possibility of reproduction. It is broader in scope and may be defined as the sum of an organism's adaptation to survival and reproduction and may include physiological and ecological considerations and resource partitioning between growth and reproduction (South and Whittick 1987). A life cycle is what actually occurs at a certain point in time as a result of prevailing environmental conditions.

culture technology, became reproductive and produced tetraspores four years after being planted into the wild. *Kappaphycus* has been farmed in different parts of the world by vegetative propagation. This is done by breaking of and planting large pieces of individual plants (SEAFDEC 2003).

Ask *et al.* (2003a) reported that, of all the introductions of *K. alvarezii* made into different parts of the world, only on two occasions was any form of quarantine procedures employed before release into the marine environment. The first instance was when *K. alvarezii* was introduced into the Solomon Islands in February 1988 (Smith 1991) and the second one was when it was introduced into Brazil in 1998 (Oliveira and Paula 2003, Ask *et al* 2003a). In both occasions the procedures employed were different.

In the Solomon Islands, when seaweed were first brought in from Fiji in 1988, they were placed in raceways at ICLARM (name now changed to WorldFish Centre) for 14 days before out transplanting. Smith (1991) reported that "the purpose of quarantine was to thoroughly clean the weed of any invertebrates which might have been present rather than prevent the spread of infectious disease". Although there was a reported increase in growth from 14 to 17 kg, during the quarantine period, the seaweed were reported to "lose condition somewhat", it suffered from necrosis and morphological changes (Smith 1991). Smith reported that in view of the absence of any recorded disease, latter shipments were kept in quarantine period for less than 14 days.

The quarantine procedures employed in Brazil on the contrary were quite stringent. Two and a half grams (2.5g) of seaweed was grown in laboratory conditions and then propagated *in vitro* for ten months to obtain unialgal cultures. Following laboratory propagation, 20 batches of 3.0 grams were transplanted monthly into a protected bay where they were planted on a floating raft. This was done over a four-year period between 1996-1999.

Ask *et al*. (2003a) also reported that quarantine procedures were employed when seaweed was introduced into Madagascar from Zanzibar, Tanzania in 1998. Only visibly clean plants were obtained from Tanzania and flown to Madagascar (Ask *et al.* 2003a). At Madagascar the seaweed were placed in aerated tanks containing seawater filtered at 5 and 1 μm. Seaweed were maintained in the tanks for two weeks. Visual inspections using a magnifying glass (5X) were made twice weekly to monitor for growth of macroalgae and animals. Water was changed twice per week and wastewater treated with chlorine bleach for 24 hours at a dose of 125 ml m^{-3} before being poured onto the ground 500m from the coastline. Plants were outplanted to test farms at the end of two weeks where they were continually monitored for any environmental impacts. Ask *et al.* (2003a) further reported that there was no environmental impacts or spread of seaweeds in the area that they were planted.

There is anecdotal evidence of quarantine procedures being applied in other places and on other occasions, for example when Fiji imported plants from Tonga in 1984 the plants were held in raceways on Makogai Islands for 2 weeks (Mario pers. comm, 2003.). A problem reported with this quarantine procedure was that thalli became necrotic so very little of the plant material was fit for re-planting. From an initial 120Kg of seaweed at the start only 14 Kg was left when the quarantine period ended (Mario *pers comm*, 2003).

Glen Joseph (*pers comm*, 2003) reported that quarantine process was applied when seaweed were introduced from Kiribati into Marshall Islands in 2002 and also during inter-island introductions within the Marshall Islands in 2002 and 2003 2003). The method of quarantine however was not mentioned. Pakoa (*pers comm*, 2003) reported that when seaweed was introduced into Vanuatu in 1999, it was not subjected to any strict form of quarantine process. They were merely being kept in hatchery tanks for 10 days before transplanting onto reefs.

Quarantine procedures were not employed when *K*. *alvarezii* was introduced to Indonesia, Tanzania and French Antilles (Ask *et al.* 2003a) because of the perception that it posed not threat. Although K. *alvarezii* has been considered benign, non-invasive and has not been known to be afflicted by any infectious diseases, researchers above (Smith (1991), Ask *et al.* (2003) and Oliveira and Paula (2003)) recommend that quarantine measures be employed when *K. alvarezii* is introduced to a new location. The main issues arising from K. alvarezii introductions are threefold, 1), possible carriers of diseases, 2) as vector for other species (which may be invasive or carriers of infectious disease) and 3) the possibility of *K. alvarezii* being invasive themselves.

Diseases

The only "disease" which affects *K. alvarezii*, is Ice-Ice (see Figure 1). Ice-ice is a malady that affects the tissues of *K. alvarezii* during stress (Ask *et al*. 2003a, Largo *et al.* 1995a). Normally the tissues are bleached and become necrotic. Ice-ice has been attributed to; 1) stress which results in the production of volatile halocarbons by the plant itself. This results in the necrosis of the plant tissue on the stressed area (Ask *et al* 2003a, Largo *et al* 1995, Pedersen *et al.* 1996.) and 2) infection by certain bacteria which has been considered to be a secondary effect of stress (Ask *et al.* 2003a). According to Ask *et al.* (2003a), no pathogenic agents have been noted for the commercial Eucheumoids (which includes *Kappaphycus*) in the last 3 decades and that ice-ice can be easily controlled by the prevention of stress. Ask *et al*. (2003a) further stated that ice-ice has not been recorded to spread to neighbouring native populations.

Figure 1: *Kappaphycus* affected by "Ice ice[5]" (SEAFDEC 2003).

Invasive species

The absence of any known pathogenic agents is not the only issue to be considered. Consideration must also be given to its possible role as a potential invasive species. *Kappahycus alvarezii* has not been reported as an invasive species or pest in Cook Islands, Fiji, Kiribati, Republic of Marshall Islands, Samoa, Solomon Islands, Tonga, Tuvalu and Vanuatu and where it has been introduced. At the same time no research has been carried out to determine whether it has any environmental effects.

Even though there are natural predators (siganids, sea urchin, seastar, turtles etc.) of *K. alvarezii* present in these countries, *K. alvarezii* has been able to propagate itself and persist to form natural populations in some of these countries long after farms have been abandoned. For instance, nine years after seaweed farming ceased in the Solomon Islands, propagules were surviving on seagrass beds. These were later used as seedstock for the revival of seaweed farming in 2001 (Sulu, pers.obs, 2001). What Ask *et al*. (2003a) has alluded that *K. alvarezii* was no longer present in the Solomon Islands 10 years after farming was abandoned is not true.

[5] Picture obtained from http://www.seafdec.org.ph/downloads/kappa.pdf on 14th Nov 2003.

Similarly, Russell (1983) reported that since it's introduction in the 1950's, *K. alvarezii* had posed no threats as an invasive species to coral reefs in Hawaii. Twenty years on, *K. alvarezii* is now considered a pest and major marine invasive species in Hawaii. Smith (in press) and other researchers, for example, Rogers and Cox (1999), report that *K. alvarezii* has proliferated and occupied significant parts of Kaneohe Bay and is spreading at a rate they consider alarming. Ask *et al.* (2003b) argued that the case in Hawaii is unique because *K. alvarezii* was introduced for research purposes rather than for mariculture. Hence there is a lack of market forces that could counter its spread by harvesting. In other island countries, it is being introduced for aquaculture and ultimately will be sold to generate income. Its spread will therefore be contained by harvesting and sale, hence will not be invasive (Ask *et al* 2003b).

Market forces however, cannot guarantee that *Kappaphycus* will not become an invasive species. In Fiji for example, seaweed farming is currently in decline due to poor internal marketing arrangements, while in the Solomon Islands it is still too early to predict. If we consider seaweed, as an industry that has the potential to collapse - as has happened in the past, then what happened in Hawaii should not be dismissed altogether as unlikely. Given the long history of use of Kaneohe Bay as a sewerage outlet which has also led to problem growths of endemic Hawaiian seaweed such as *Dictyota acutiloba*, it does appear however, that any threat as an invasive species would most likely be greatest in places where severe eutrophication has occurred, and would not be the only species implicated. In oligotrophic reef environments such as in Fiji's Lau Group, *Kappaphycus* has merely "persisted" rather than "proliferated".

***Kappaphycus alvarezii* as a vector for other exotic species or pests**

Kappaphycus alvarezii should not be discounted as vector for unwanted species. An example is Kiribati, where Russell (1982) reported that *Acanthophora spicifera, Dictyota acutiloba Hypnea musciformis and Ulva reticulata* were introduced with *Kappaphycus* into Fanning Island, Kiribati, from Kaneohe Bay,

Hawaii. There is also potential for a range of invertebrate or even small fish species to be harboured by *Kappaphycus* plants and be inadvertently transported to new locations. After harvest of *Kappaphycus* plants (from Kiuva and transported to USP in Fiji) during a USP class exercise in 2003, a small Moray eel was discovered among plants as they were being weighed back on dry land (Pickering, pers. comm.).

Environmental Impacts of seaweed farming

There has been very little or no research conducted on the environmental effects of *K. alvarezii* (or seaweed farming in general) on the local biota following introductions and culture. Johnstone and Olafsson (1995) and Olafsson *et al*. (1995) are among the few studies that have been done. Johnstone and Olafsson (1995) investigated the population dynamics of benthic meiofauna, the primary and bacterial production in the associated water column and the benthos, and the flux of nutrients between the benthos and water column in farmed and non farmed control areas. They reported that seaweed farming has no discernible effects on water column microbial production, but has a clear effect on benthic microbial process and meiofaunal populations. While they were not able to specifically identify which aspects of algal farming was responsible for changes, Johnstone and Olafsson (1995) however hypothesized that the mechanical alteration of sediment surface and enhancement of local benthic fish grazing may have played a role.

Olafsson *et al*. (1995) assessed the effects of seaweed farming on benthic communities. They reported that major meiofaunal taxa were found in significantly lower numbers within farmed areas compared to non farmed control areas. They concluded that increased predation by benthic feeding fish and mechanical disturbance of sediments was a possible cause for the differences in meiofaunal abundance between farmed and non-farmed areas.

A recent report by Zemke-White (in prep) commissioned by Conservation International (CI) on **Environmental Impacts of Seaweed Farming in the Tropics** presents some alternative views. He points out that the paucity of literature on impacts of seaweed farming is mainly attributable to: 1) lack of funds for what often needs to be an extensive study, and 2) the fact that from an environmental perspective seaweed farming is considered a benign or even positive form of marine agronomy. Zemke-White (in prep.) listed the various intuitively-appealing factors cited by proponents of seaweed farming (for example, Ask (1999)) which are:-

(1) Farms act as nutrient sinks

(2) As farms are a site of both primary production and herbivory, they can act to enhance fish stocks

(3) Farms can increase the available habitat for certain fish and invertebrates

(4) Farming can provide a sustainable livelihood which may take people away from more destructive activities like dynamite fishing or cyanide fishing

(5) As farms require a certain standard of water quality, the farmers will develop a sense of stewardship toward the coastal area and will influence people whose activities are a threat to water quality.

Zemke-White (in prep.) presented some counter arguments regarding the above claimed benefits. Farms acting, as nutrient sinks may be beneficial in eutrophicated waters, however this may have a negative affect on most reef environments, which are generally nitrogen, limited. Nitrogen will also be lost from the reef food web through the harvesting of seaweed and may not be available for other organisms. Seaweed breakage and loss from farms, which may eventually find their way into the food web, however may offset nutrient loss (through seaweed harvest) from coral reefs. Although, no studies have been conducted to assess whether such nutrient loss from the environment is real or

merely hypothetical. Zemke-White (in prep) however cites anecdotal evidence from the Philippines where farmers reported non-productive farms after a period of 4-5 years. Productivity was only possible after a fallow period of two or more years.

With regards to seaweed farms acting as areas of primary productivity, Zemke-White (in prep) argued that there is a possibility that seaweed may actually lower productivity by inhibiting growth of micro-algal mats. Furthermore, even though primary productivity may be high per unit area, this productivity does not contribute to the reef as it is removed during harvesting. Seaweed lost through breakage and loss may however offset this loss of productivity. Similar to nutrient loss, no studies have been conducted to quantify such loss. Seaweed farms are also postulated as important habitats for fish and invertebrates, the question however postulated by Zemke-White (in prep) is; *"what happens to these organisms once seaweed is harvested?".* Zemke-White (in prep) argued that; *"while there may be an increase in invertebrate diversity on the farms, if all these invertebrates are then harvested along with the seaweed, the increase to the local community at large may not eventuate*".

The contribution of Seaweed farming toward prevention of destructive fishing practices and promotion of stewardship by farmers over the sea may also be not necessarily true. According to Zemke-White (in prep.) seaweed farming may be just one of the income generating activity a fisher engages in, besides other activities, one of which for example may be dynamite fishing.

There is very little results from rigorous scientific study available from which to convincingly conclude either that *Kappaphycus alvarezii* will or will not pose a risk of adverse environmental effects upon introduction to a new locality. Certainly environmental changes from seaweed farming have been documented but whether these can be considered "adverse impacts" is a matter of scale and a matter for conjecture. Arguments can be made either way but the issue is still

in the realm of speculation and the main body of available evidence is largely anecdotal. In addition, the answer will be different from case to case, especially between oligotrophic and eutrophic environments. The possibility thus remains that *Kappaphycus* can have both positive and negative impacts. The general perception that this is one of the more benign types of exotic-species introductions to make is probably correct, however it cannot be claimed that it is entirely without risk. The decision to import *Kappaphycus alvarezii* or not will therefore be a sovereign decision for each country to make, after considering a range of economic and environmental factors and weighing them up. According to Nash (pers.comm. 2003), if a country is a signatory to a convention on exotic species, it may be constrained in the decisions it can make. The question then arises – what is the best practice to follow when introducing this seaweed to a new locality?

The Secretariat of the Pacific Community proposes the following quarantine procedures when introducing *K. alvarezii* to a new location. These protocols are probably based on the protocols proposed by Ask *et al.* (2003a.) Ask *et al.* (2003a) reported that these protocols and the introduction procedures for cultivation were designed by considering the FAO-Code of Conduct for Responsible Fisheries (1995) and the FA0-Technical Guidelines for Responsible Fisheries (1996). The quarantine procedures are shown below.

SPC *Kappaphycus* Seaweed Quarantine Protocol

Pre-export

- Seaweed propagules should be selected from the young healthy portion of the plant and are free of epiphytic algae
- Minimal quantities of seaweed are to be selected (10-30 kilograms)
- The surface of propagules is free of sediment, macro fauna and flora (ie any entangled drift seaweed)

Notification

- The respective quarantine authorities of the importing country are to be notified in advance of transhipment
- Airline and freight agents are to be notified that the shipment contents contain live plant specimens

Quarantine facilities

- Seawater supply is pre-treated by filtration through 1 micron sieve
- Seawater is from a source with sufficient nutrient levels (preferably not oceanic water)
- Seawater salinity is at least 28 parts per thousand
- Seawater temperature is stable and in the range of 25-30 ^{o}C
- Aeration is provided to generate adequate water flow
- The seaweed quarantine unit is isolated from other aquaculture facilities
- Access to the quarantine facility is restricted to authorised personnel only
- All other fauna or flora to be excluded from the quarantine facility
- The seawater outflow is discharged into a sump pit which is out of range of the high tide water mark, at a location that can safely treated with herbicide
- Equipment used in the quarantine facility, such as scrubbing brushes, thermometers, filters and etc, are to be treated with a chlorine dip after use
- Holding tanks are to be drained and scrubbed clean at least twice a week

Treatment

- Upon arrival the seaweed is to be thoroughly rinsed with fresh seawater before placement into holding tanks
- Seaweed stock are to be held under quarantine for at least two weeks
- Seawater in the holding tanks are to be changed twice per week
- Discharged water is treated with chlorine bleach for 24 hours at 125 ml m^{-3} dose
- Stress of seaweed stock is to be minimised
- Seaweed are to be visually examined by hand daily for unusual signs
- Seaweed samples are to be sacrificed for a surface microscopic examination using a magnifying glass (5x) for signs of epiphytes
- A daily log to be kept, recording details of treatment, observations and clinical abnormalities

Criteria for not releasing imported seaweed into the local environment

- The presence of unexplained flora or fauna associated with the seaweed
- Unexplained unusually high mortality of the seaweed
- Unexplained lesions on the seaweed
- Fungal infections on the seaweed
- Suspicion that non-endemic organisms associated with the seaweed may be introduced into the wild

Ecological monitoring

- Prior to out planting a baseline survey of species biodiversity is to be conducted within an area of 0.5 kilometres vicinity from the proposed farm site
- Upon placement the seaweed are to be visually examined for abnormal signs of stress and mortality
- The location of the seaweed is to be surveyed to see if the site is host to any unusual parasites
- An area of 0.5 km vicinity surrounding the seaweed farm is to be monitored over a 1 year period for signs of unusual ecological disturbances or of loose seaweed becoming established in the wild in significant quantities.

Presented below are results of a trial of the quarantine protocol proposed by SPC and tested by the Institute of Marine Resources at the University of the South Pacific. The aims of the trial were:-

(1) To test whether the protocol can isolate and eliminate organisms that may have arrived with the seaweed samples, with a view to reducing the risks of introducing exotic species.

(2) To observe for any possible infections that may affect the seaweed during the quarantine period.

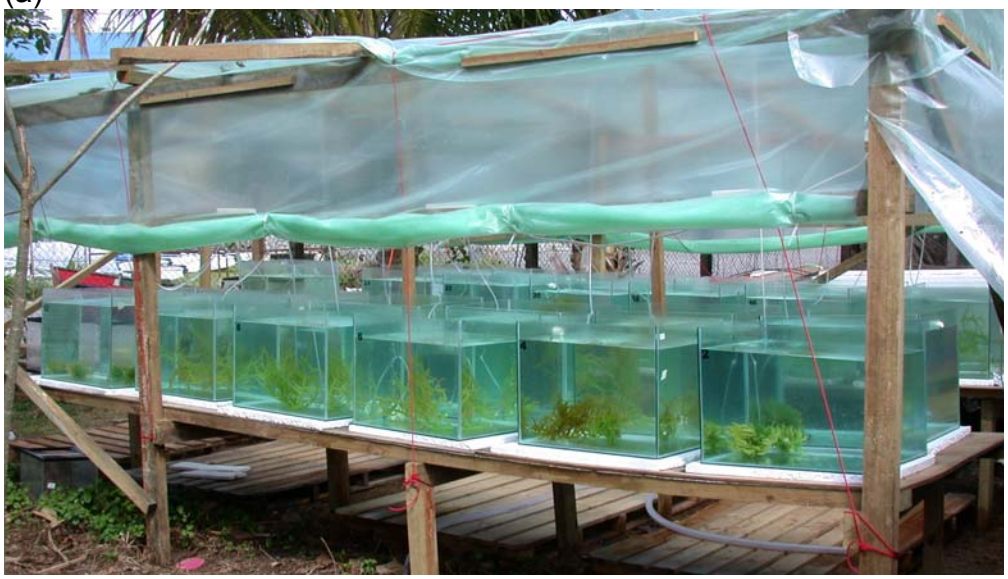
(3) To observe the health status of seaweed during the quarantine period. Since the seaweed were destined for mariculture, it is important that they remain healthy till the end of the quarantine period. The growth rates, wet weights and physical appearance of the seaweed were used as indicators of seaweed health.

Experimental set up

An open-walled timber shed was constructed to hold the seaweed tanks for the experiment (see Figures 2a-c). A transparent plastic was used to shelter the top of the shed to protect the seaweed from rainwater. The plastic covering was transparent enough to ensure ample natural light was available for the seaweed to photosynthesise.

Figure 2: (a-c) The open-walled timber shed used for quarantine experiment.

(a)



(b)

(c)

Field collection

Samples of seaweed – *Kappaphycus alvarezii* (var. tambalang) were collected by field staff of the Fiji Department of Fisheries from three farms on the northern island of Vanua Levu in Fiji - Macuata, Savusavu and Bua. At each location, after collection, the seaweed were wrapped in wet mutton cloth, placed inside polystyrene boxes and transported by air to Suva. The seaweed arrived at USP in less than ten hours of being collected from the field and all appeared very healthy and in very good condition (see Figures 3a & b).

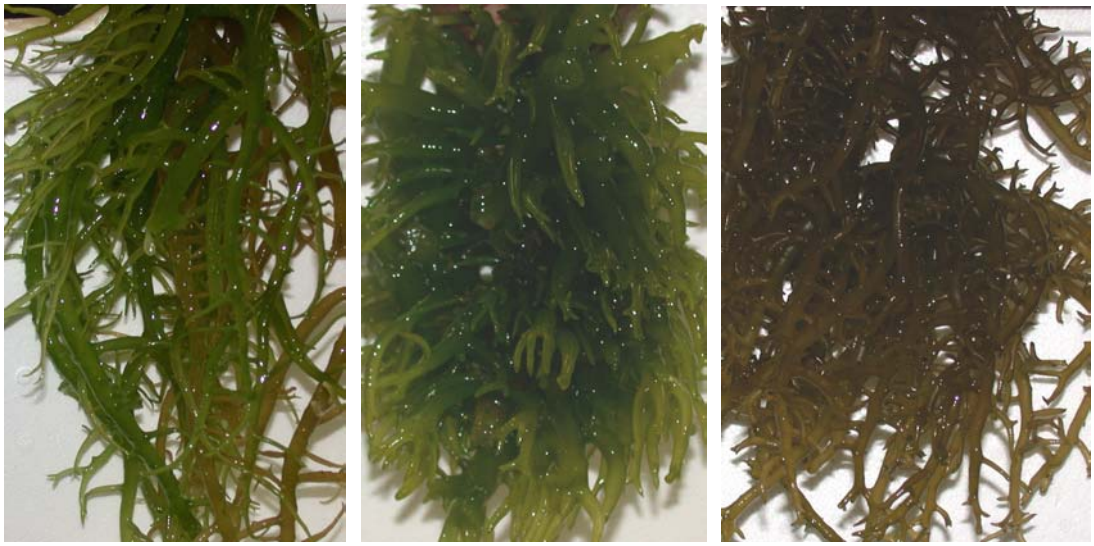
Figure 3: (a) Seaweed from Bua sent in polystyrene boxes (b) Excellent condition of the seaweed on arrival.

(a) (b)

Although the same variety of *Kappaphycus alvarezii* (var. tambalang) was being farmed in Bua, Macuata and Savusavu, the morphology and appearance of the seaweed between locations was quite different (see Figures 4a–c and Appendix 1). This seaweed, in common with other red seaweed, is morphologically quite plastic in response to environmental conditions. For instance, the seaweed from Bua were compressed, ball-like, quite chunky or fleshy, and occurred in shorter strands/branches as compared to those from Savusavu and Macuata which were longer and slender and could be easily broken. The Bua seaweed were more robust because the farm is located in an area where there are stronger currents and relatively more wave action than the other two sites (Mario, *pers comm*m; 2003).

Figure 4: The different morphologies of *K. alvarezii* from (a) Macuata (b) Bua and (c) Savusavu farms.

(a) Macuata (b) Bua (c) Savusavu



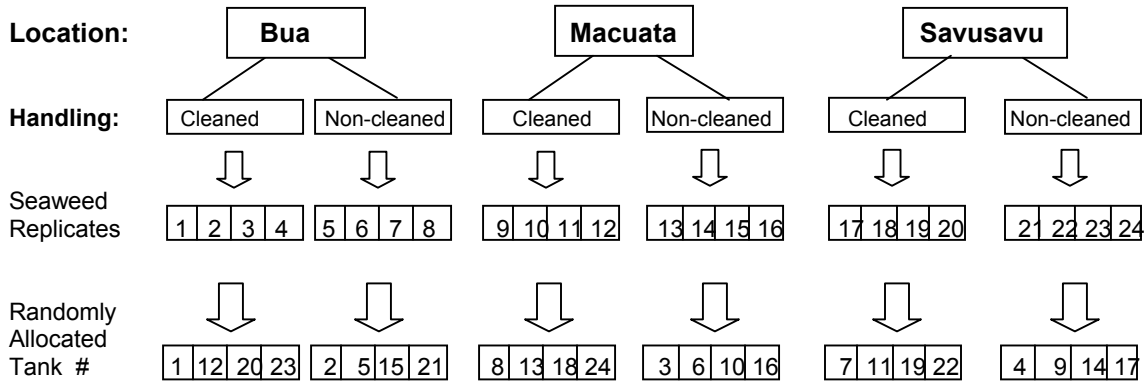
Experimental methodology

Upon arrival at USP, the seaweed from each location was divided into two sets. One set was thoroughly washed with fresh seawater and manually cleaned of any visible epiphytes or drift algae and epifauna. The other set was only rinsed in fresh clean seawater; they were not handled to remove associated organisms. During the weekly cleaning sessions in quarantine, the former set was given a rinse and thorough cleaning while the latter was given a rinse only. The wastewater from the initial cleaning/rinsing of seaweed from each location was collected, washed through a 250 μm sieve and the residue stored in 70% alcohol stained with 10% Rose Bengal solution. These were later examined under the microscope for any organisms.

About 500-700g (for a stock density of 5-7 grams per litre) of the seaweed propagules from each set were weighed and randomly allocated to 24 static glass aquarium tanks (dimensions: 59 cm x 44 cm x 39 cm). Four replicate tanks were kept for each treatment (see Figure 5). Each of the tanks received one micron filtered fresh lagoonal seawater twice a week. The tanks were vigorously

aerated to prevent boundary layer effects and to create circulation required for healthy growth of seaweed. Figure 6 shows a tank containing the seaweed propagules.

Figure 5: Seaweed distribution from different localities among tanks



The seaweed were subjected to the proposed SPC quarantine protocols, for a period of 16 days (September 3rd – 17th, 2003) Observations on the colouration of seaweed, physical appearance, any presence of infection or necrosis were made daily. Microscopic examinations to observe for phytal fauna and flora were done twice weekly during water change. Wastewater from the water change was stored in a tank and treated with ordinary chlorine bleach at a concentration of 125ml m^{-3}. The water was examined under the microscope 24 hours after treatment for any living organisms and then discarded.

For growth measurements, the seaweed were weighed three times: (1) at the start of the experiment – day 1, (2) a week later – day 9 and then (3) finally upon the termination of the experiment – day 16. The measurements were used to calculate the Instantaneous daily growth rates using the formulae:

$$\text{Relative growth rate}(\%\ \text{day}^{-1}) = (\log_e n_2 - \log_e n_1) \times 100/t$$

Where: n_2 = mean final seaweed weight

n_1 = mean initial seaweed weight

t = time in days

Figure 6: Glass aquarium tank used to hold the seaweed; contains air stone and a temperature logger (grey cylindrical).

Two-way ANOVA was used to determine whether location (from where seaweed were obtained) and handling stress had any significant effect on growth rates of the seaweed at the end of the 16 day quarantine period. We also performed repeated measures ANOVA on the wet weights to determine if there was a significant temporal difference in the wet weight of the seaweed during the course of the quarantine period.

Results

Seaweed biota

The most obvious result was that the seaweed samples supported few epiphytes and contained a sparse phytal fauna. Some samples yielded practically no visible biota but the washings from all samples had a diverse microscopic biota especially diatoms.

The various plants and animals collected on arrival from an initial cleaning and rinsing are shown in Figure 7(a-f). Macroalgae included fragments of drift algae including: *Hydroclathrus* sp., a species of *Amphiroa* sp. (a coralline), *Hypnea* sp and a few unidentified filamentous Rhodophyta and Phaeophyta. In general the samples yielded very few large animals. There were no crabs, small mollusca, large polychaetes or fish larvae for example. The largest animal found, in just one sample, was a solitary 3 cm long mantis shrimp (Fig 7b).

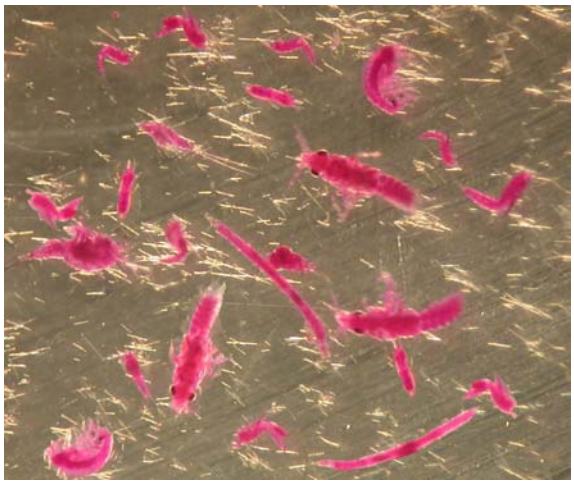
The macroscopic animals that were found included mainly copepods, isopods, amphipods and shrimps, and nematode worms. Some specimens had small sponge colonies, and what we think was a bryozoan (Fig 10a), but these tended to remain attached and were absent from the washings. Most of these macro-organisms were extracted from the samples from Bua which exhibited the most compact pattern of branching. Apart from a few drift algae, the specimens from Macuata and Savusavu did not contain any macroscopic organisms. It is important to note here a weakness in the experiments, that where the incidence of infestation by a particular taxon is low, it is unlikely that the number of replicates (N=4) is enough to detect them and may require very large samples. However it demonstrates that with a transfer of small amounts of seaweed it reduces the risk of transfer of organisms.

Microscopic examination of the washings that passed through the 250 µm filter revealed that all specimens yielded phytoplankton (especially diatoms) and

zooplankton. Protozoa and bacteria were undoubtedly present in the washings, but we made no microscopic examination to that size level. We noted that preservation with 70% ethanol destroyed most of the dinoflagellates and other naked flagellates. So re-examination of preserved samples will underestimate these life forms. Treating the washings that passed through the 250 µm filter with domestic bleach (12.5% volume to volume) killed all life forms in the washings within 24 hours.

Figure 7. Animals and plants found in the *Kappaphycus* samples on arrival (a & b) various invertebrates (c-f) miscellaneous algae.

(a) Array of animals (5X)



(b) Mantis shrimp

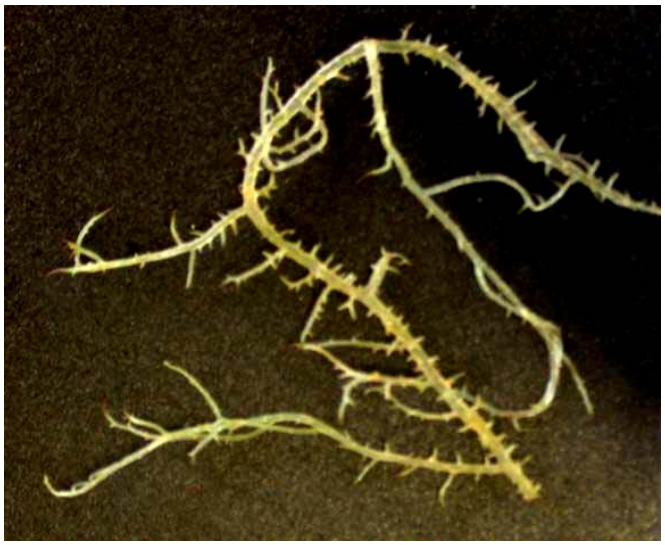
(c) *Hydroclathrus* sp.

(d) *Amphiroa* sp.

(e) Fragments of seaweed (*Hypnea* sp? or *Acanthophora* sp?)

(Figure 7 continued)

(f) *Hypnea* sp. found on *Kappaphycus* on arrival



At the end of the quarantine period, there were no macroscopic organisms larger than 1 mm present on the thoroughly cleaned seaweed, except for sporadic growths of a filamentous brown alga (possibly in the Sphacelariales) up to 1 mm long which was observed in most samples (Figure 8). We suspect that these plants would have become quite large and possibly overgrown the *Kappaphycus* given time. Another persistent epiphyte was a filamentous red alga, possibly a species of *Neosiphonia*, which embeds itself firmly in the thallus (Figure 9). This epiphyte was mainly found in specimens from *K. alvarezii* (var. *sacol*) from Kiuva in Viti Levu which as mentioned above may be a different species of *Kappaphycus*.

Figure 8: Epiphytic filamentous algal growth (*Sphacelaria* sp.?) on seaweed.

(Magnified 25X)

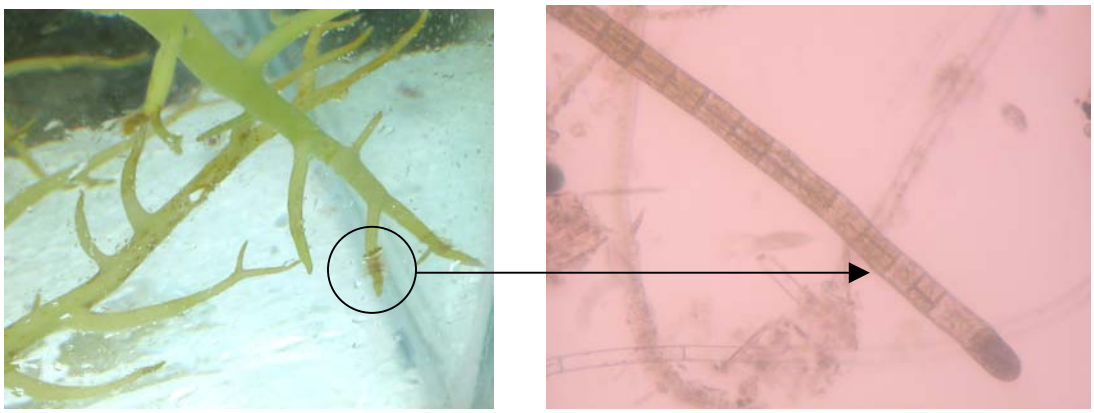
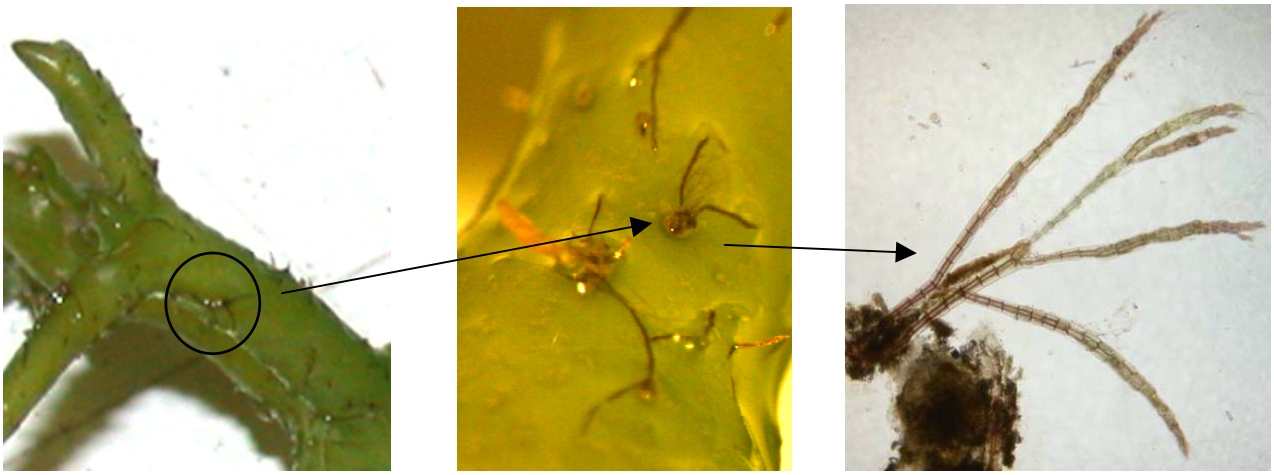


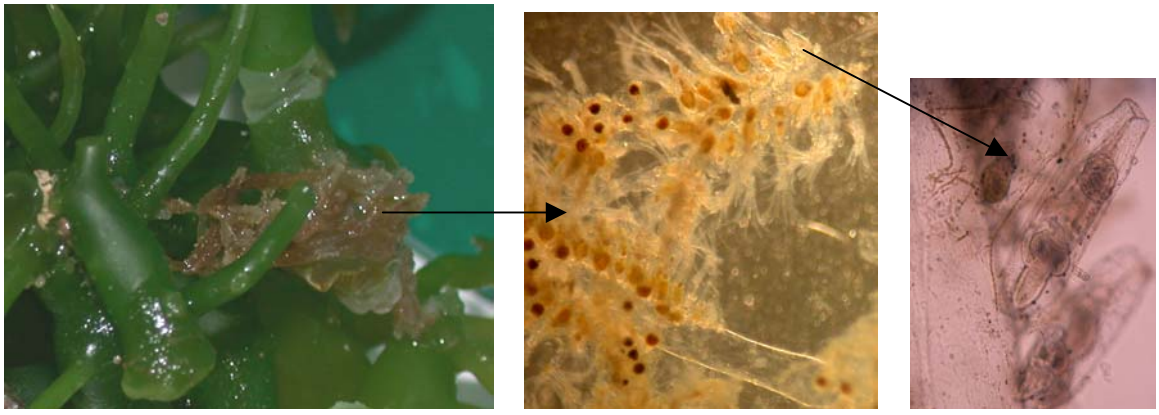
Figure 9. Epiphytic algae (*Neosiphonia* sp.?) embedded into thallus of *K. striatum* (var. sacol) from Kiuva in Viti Levu.



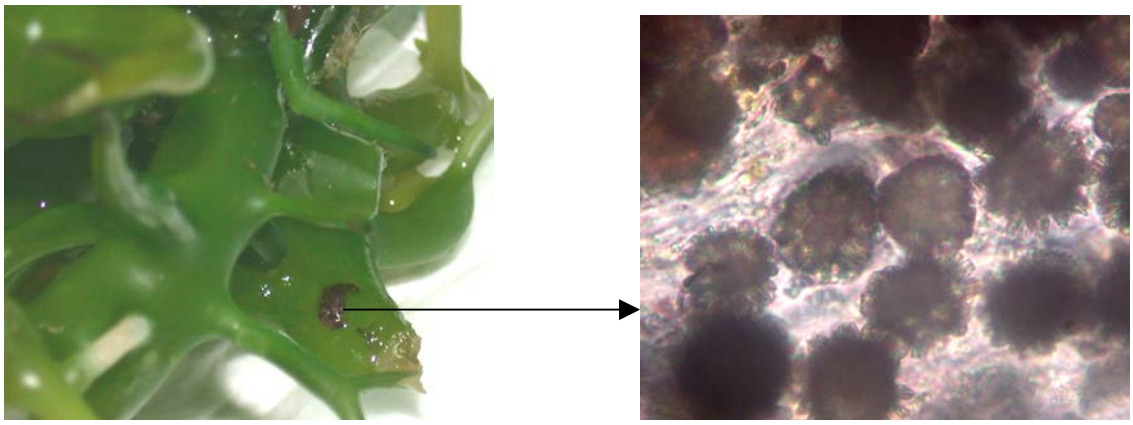
Some of the rinsed only seaweed, particularly those from Bua harboured some organisms (See Figure 10a - c) which we could tentatively identify as zooanthids, sponges and calcareous egg masses? or bryozoans?. We were not able to confirm the identification of these organisms.

Figure 10. Different types of animals found on *Kappaphycus* (unclean Bua samples) at the end of the quarantine period.

(a) Zooanthid? 10x magnification 40x magnification



(b) Sponge? 25x magnification



(c) Calcareous egg masses or Bryozoans?

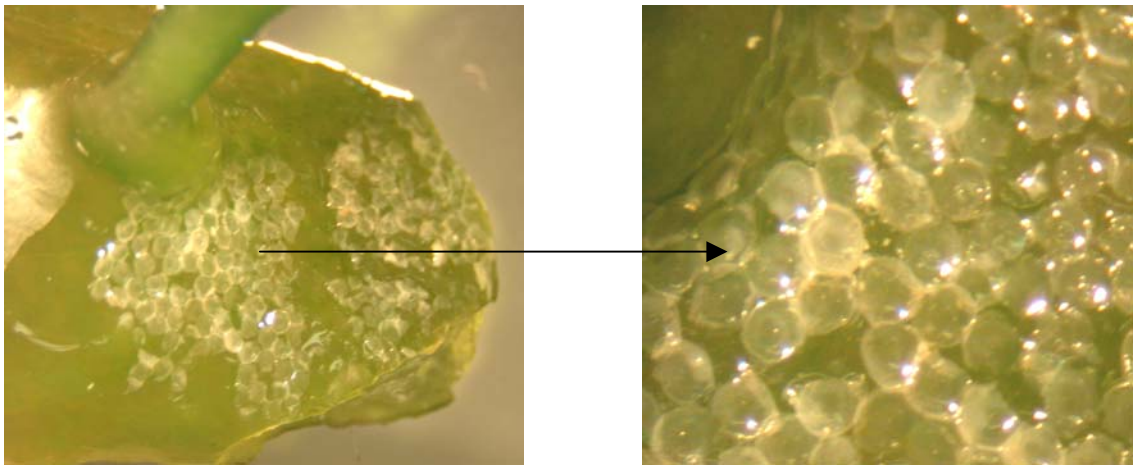
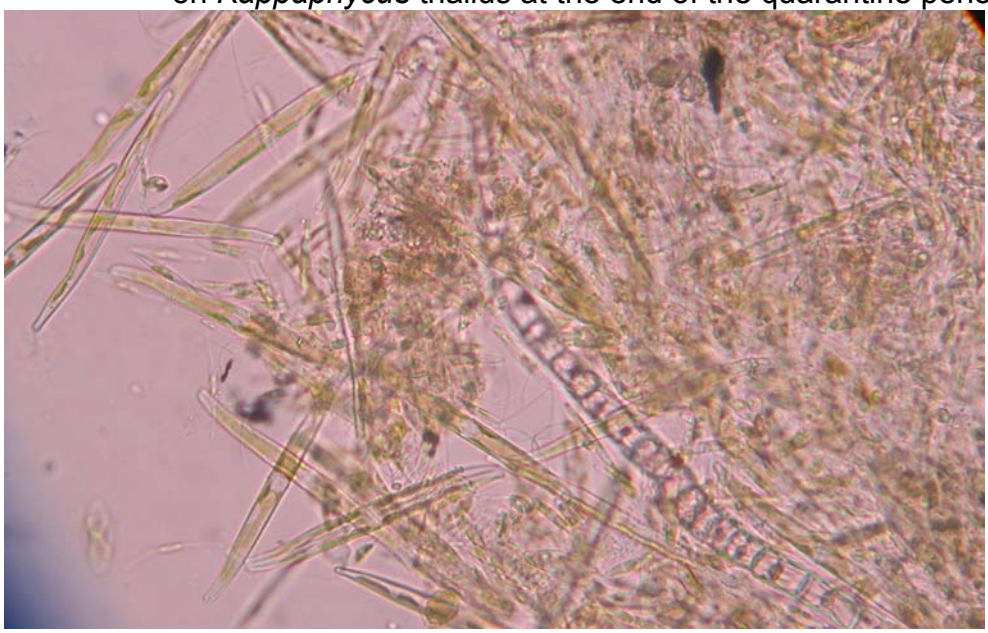


Figure 11. Myriad of microorganisms (Diatoms, Cyanophytes, spores etc) found on *Kappaphycus* thallus at the end of the quarantine period- (25X)



Microscope examination of the sacrificed *K. alvarezii* (both from the thoroughly cleaned and rinsed-only seaweed) revealed a plethora of micro-organisms on the surface of the seaweed thalli. Many of these were types we had previously seen when we first examined the washings that passed through the 250 µm sieve and which were killed with domestic bleach. These included diatoms, dinoflagellates cyanophytes and other filamentous microalgae (see Figure 11).

General observations of seaweed during the 16 days quarantine period

The appearance of the seaweed in each treatment for days 1, 5, 11 and 16 is given in Appendix Two. The seaweed generally remained healthy during the quarantine period. General observations are summarized below (Table 6).

Table 6: Summary of general observations made on the seaweed during the quarantine period.

Treatment	Summary
Macuata non-clean	All 4 replicates (T3, T6, T10, T16) healthy in week 1. Day 7 – epiphytic filamentous algae appeared in T6 covering about ~10% surface area of seaweed (See Figure 7). Day 9 – seaweed in T10 & T16 began to develop necrosis on their thallus and tips (ice-ice). The thallus colour became paler than initial colour.
Macuata cleaned	All 4 replicates (T8, T13, T18, T24) healthy in week 1. Day 7 – T13 seaweed began showing white tips. T18, T8, T24 – began developing necrosis from Day 9. By day 16 – all seaweed had necrotic thallus, and began to show tissue loss.
Savusavu non-clean	All 4 replicates (T4, T9, T14, T17) healthy up to day 10 though showing some loss in colour (paler). By day 14 T9 & T14 severe necrosis in some parts of thallus; by day 16 T17 and T4 remained healthy (though paler), T9 and T14 appeared very necrotic and unhealthy.
Savusavu clean	All replicates (T7, T11, T19, T22) healthy in week 1. T11 and T7 remained healthy throughout quarantine. T19 began showing necrosis on few tips on day 10 and 3% of tips had necrosis by day 16. T22 – started showing necrotic tips by Day 8 and by day 16 was severely affected by necrosis (70%).
Bua non-clean	All replicates (T5, T15, T2, T21) generally healthy in week 1. Day 7 – few necrotic tips appearing in T21, by day 16, 5% of tips affected by necrosis and few lesions. T2 healthy till day 16, some loss of colour seen. T15 recorded good growth and was very healthy though base thallus showed degeneration (Figure 12a) and some epiphytic filamentous algae was observed on seaweed on day 12 (Figure 12b). T5 – healthy throughout quarantine, showed good growth, some necrosis on thallus noted.
Bua clean	All replicates (T1, T12, T20, T23) generally healthy in week 1. All showed loss in colour. By end of week 2 light tips on T20 (6%), T23 (4%), T12 (end of basal thallus became diseased showing red colour and some rotting), T1 remained healthy throughout quarantine.

Figure 12: (a) End of basal thallus showing infection and degeneration and (b) Epiphytic filamentous algal growth on *Kappaphycus* from Bua.

(a) (b)

Irrespective of treatment, the thallus tips of some specimens became necrotic after day 7 and began to decay after day 12. The decay then spread towards the base of the plant. In general, however, most specimens irrespective of treatment remained healthy although some loss in colour was noted in the second week. Epiphytic filamentous algal growth (see Figure 8 and Figure 12) began appearing from day 7 mainly on uncleaned specimens but also to a lesser extent on specimens that had been thoroughly cleaned on day 1 and twice weekly thereafter.

Microscope observations of wastewater collected from the tanks during water changes showed no large organisms (larger than 1000 μM), but a rich microflora of diatoms and cyanophytes, plus fragments of the presumed *Sphacelaria* sp (see Figure 11). Once again addition of domestic bleach (12.5% v/v) eliminated these organisms.

Growth rates

The seaweed generally showed signs of good growth in quarantine. Analyses of variance showed that there was no significant difference in the instantaneous daily growth rates (% day^{-1}) of seaweed from Bua, Savusavu and Macuata (p = 0.351), (Table 7). There was however, a significant difference (p = 0.043) in the growth rates of seaweed that received thorough cleaning and those that were just rinsed, with the thoroughly cleaned specimens growing at a significantly slower rate (Table 7, Figure 13).

Table 7: Results for two-way ANOVA on the effects of location and handling on growth rates of seaweed at the end of the quarantine period. Levene's test for equality of variances at p =0.05 was non-significant (p = 0.062)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Location	0.0434	2	0.0217	1.112	0.351
Handling	0.0926	1	0.0926	4.742	0.043
Location*Handling	0.0289	2	0.0144	0.741	0.491
Error	0.352	18	0.0195		
Total	1.428	24			

All specimens steadily gained weight during the quarantine period. On day 16, the average weight for specimens that received minimal handling (rinsed only) increased by 10%. However, the relative growth rate of those specimens that were thoroughly cleaned, increased by just 4.9%.

Repeated measures ANOVA (Table 8) showed that the two handling treatments (thoroughly cleaned versus rinsed) versus had little or no effect on growth expressed as an averaged increase in wet weight (p = 0.402). In Figure 14 the two line plots are not significantly different. Time however, had a significant effect on the wet weights (p= 0.000) with growth rates probably slowing with time. Scheffe's post hoc comparisons showed a significant difference between the wet weights taken on day 1 and day 9 (p = 0.00) and day 1 and day 16 (p = 0.00),

whilst no significant difference was noted between the wet weights of day 9 and day 16 ($p = 0.452$). (If the plants had been weighed daily then the plots in Figure 14 would have probably been a flattening growth curve).

Figure 13: Effect of cleaning treatment on the growth rates of seaweed

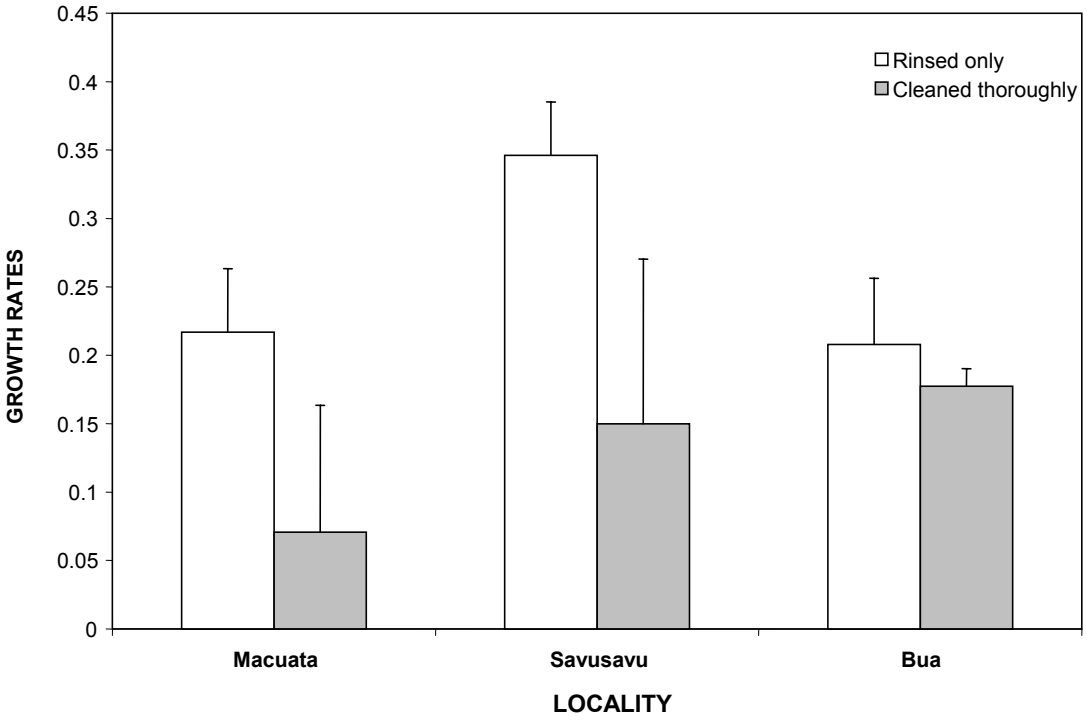


Figure 14: Average wet weight of seaweed under the two handling regimes over time

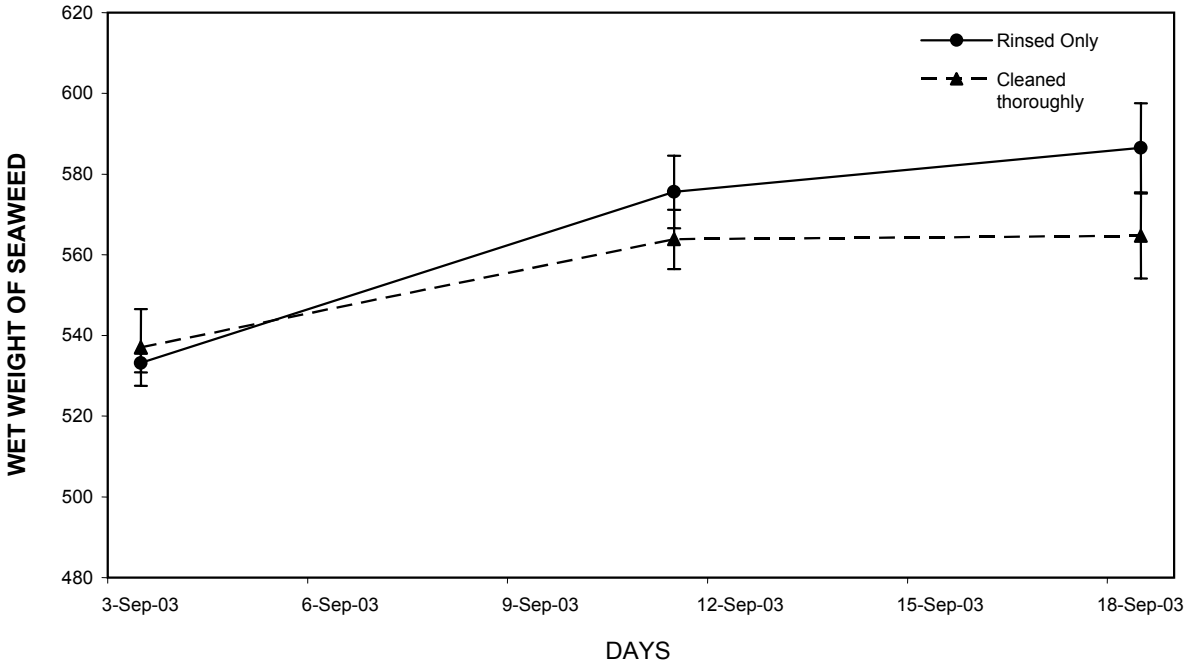


Table 8: Results for repeated measures ANOVA on the effects of time (within subjects – 3 levels) and handling (between subjects) on the wet weights of the seaweed taken during the course of the quarantine period; at 95% confidence level.

	df Effect	MS Effect	df Error	MS Error	F	p-level
Time	2	11478.8	22	253.668	45.25079	.000000
Handling	1	1764.68	11	2327.626	.75814	.402513
Time* Handling	2	1005.42	22	352.077	2.85568	.078961

Diseases and lesions

Seaweed began showing signs of stress and necrosis by the end of the first week of quarantine. Almost all seaweed developed some level of necrosis by the end of the experiment. Some were severely affected, while others showed just a few necrotic tips. (See Figure 15 a & b). Although all seaweed recorded growth, the colour of their thallus was noticeably paler at the end of the experiment. An example is shown in Figure 16 a & b for a seaweed sample from Bua.

Figure 15: (a) Seaweed severely affected by necrosis and (b) seaweed less affected by necrosis

(a)

(b)

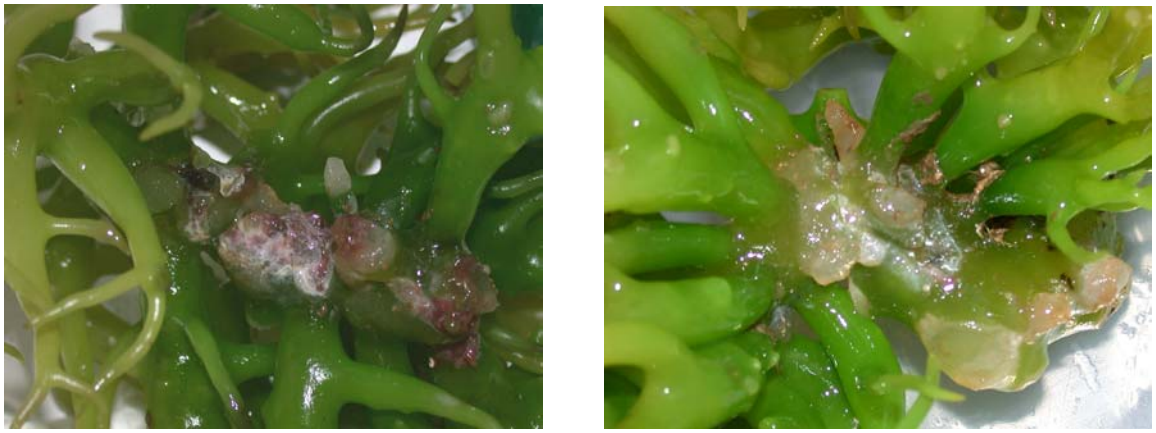
Figure 16: a) Healthy dark coloured thalli on arrival b) Paler thalli at the end of the quarantine period.

(a) (b)

Some seaweed developed lesions/infections on their thallus ends, which were injured when it was broken off from bigger seaweed bunches. See Figure 17 a & b). Some lesions (e.g. in Figure 17 c & d) were unexplained, probably bacterial and or fungal infections. The infected lesions spread over the immediate area and remained unhealed till the end of the experiment.

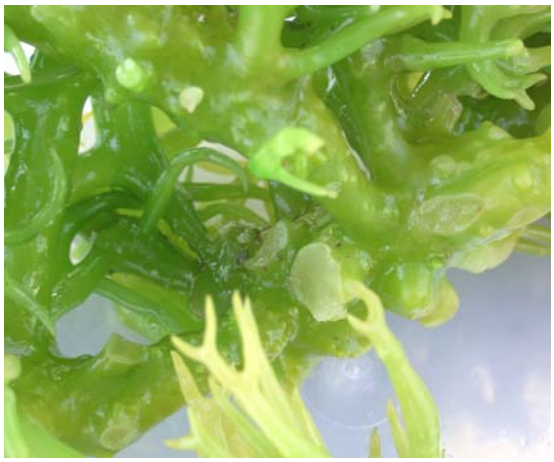
Figure 17. a) Infection at the broken end of seaweed, (b and c) Unknown lesions, (d) Bacteria/fungal infection on the seaweed.

(a) (b)



(Figure 17 continued)

(c)



(d)

Discussion

Quarantine is about reducing the risk of species or diseases becoming problematic in new locations. In the case of transplanting *Kappaphycus* to new locations, it is about reducing the risk of the seaweed itself becoming a pest and about reducing the risk of introducing associated species such as epibiota and seaweed diseases. The protocols tested here are not designed to minimise the risk of *Kappaphycus* becoming an invasive seaweed and possible pest. However, because the seaweed has previously been transplanted to several locations where it has not become particularly invasive or a pest, that risk may be low. The risk of introducing other species with *Kappaphycus*, especially mircoorganisms is, however relatively high unless some precautions are taken.

Compared with many other seaweeds, *Kappaphycus* has a relatively sparse phytal fauna. Many seaweeds, species of the brown seaweed genus *Sargassum* for example, contain myriads of animals in high numbers that soon become apparent when the seaweed is immersed in water to which a little formalin has been added. Some species of the green seaweed genus *Codium* are heavily infested with millions of nematode worms living interstitially amongst the hyphal-like strands of the alga. Many turf-like algae, that trap sediment, are packed with crustaceans, small molluscs, worms and so on. In comparison, *Kappaphycus* is a relatively clean seaweed.

The density and diversity of the phytal flora and fauna associated with *Kappaphycus* depends to a large extent on its morphology which, like many seaweeds, is highly variable. Some specimens, such as those from Macuata being relatively long, slender and streamlined, offer less habitats for associated marine life. Other plants, such as those from Bua are more finely divided and compact, thereby offering few nooks and crannies for phytal flora and fauna. These morphological differences explain the relatively high abundance and

diversity of phytal flora and fauna that was washed out of the Bua specimens compared with plants from other localities.

This simple observation immediately presents an opportunity for reducing risk. When translocating *Kappaphycus*, choose streamlined specimens with minimal branching as the transplants. Then it is relatively easy to wash away the phytal fauna which is unlikely to be very abundant in the first place.

Our results have shown that washing the seaweeds with filtered seawater to remove macro and micro-biota reduced the incidence of such organisms on the transplanted fragments over a two-week quarantine period. But such washing treatment will not eliminate all the organisms, especially epiphytic seaweeds that may be embedded in the tissues of the *Kappaphycus* and the plethora of diatoms and other microbes on the surface of the plant. Nevertheless the washing treatment is a useful step that will further reduce the risk of introducing associated species. The procedure comes with a cost however, because we found that the handling and mechanical damage caused by the washing and rinsing caused those cuttings to grow more slowly than others which had been left alone and untreated.

Another important factor to consider is whether to transplant entire plants or parts of plants. Since *Kappaphycus* is readily propagated by fragmentation it is unnecessary to transplant entire specimens. This confers another means of reducing the risk of introducing associated species. Most seaweeds grow from apical or basal meristems, ie their youngest tissue is respectively near the tips or at the base depending on the species. For kelps such as species of *Laminaria*, which have basal meristems, the oldest tissues are at the tips of the blades where the kelp tissue is degenerating and sloughing off. It is on these tissues that the richest epibiota is found and where densities of microbes are highest. Near the basal meristem, however, just above the stipe (stalk), the meristematic tissue is visually clean and microscopic examination reveals the surface to be relatively

free from fungi, bacteria, protozoa and other epibiota (Hay *pers obs* on South African *Laminaria* and *Ecklonia*). So if one were to transplant *Laminaria* sporophyte plants, and want to reduce the risk of introducing associated species, then that would be best achieved by cutting off most of the blade and transplanting the holdfast, stipe and basal meristem.

For seaweeds with apical mersitems, like *Kappaphycus,* the reverse applies. The oldest tissue is at the base of the plant. This tends to be the region where most epiphytic algae are attached. In many brown seaweeds, species of *Cystophora* for example, epiphytic algae, calcareous tube worms, hydroids, bryozoans colonial tunicates, sponges and so on are invariably attached to the base of the plant. The tips of the branches are relatively free from epibiota. This is presumably because this actively growing region produces various chemicals and mucus-like substances that act as antifouling substances. Brown seaweeds produce tannic acid for example, while many red and brown seaweeds copiously release polysaccharides. So when transplanting *Kappaphycus,* a sensible quarantine procedure would be to select tips of branches in preference to exporting entire plants.

The volume of material that is transplanted is also an important factor to consider. The risk of introducing hitchhikers and other associated species undoubtedly increases with the volume of seaweed being shipped and the risk-to-volume relationship may be logarithmic!

The smallest propagules that can be transplanted are spores. Ideally *Kappaphycus* should be translocated as spore solutions, with the spores being germinated in culture at the new location. However, sexual specimens of *Kappaphycus* are uncommon, and considerable skills, experience and laboratory facilities are needed to identify the reproductive plants, to induce them to reproduce and to provide “seed” for transport to a new location. At the new site similar expertise and equipment is needed to germinate the spores and cultivate

and on-grow the germlings. Realistically, translocations of *Kappaphycus* in the Pacific will invariably be by vegetative propagation.

If the transplanted “cuttings” were reduced to just a few well rinsed fragments each just a few cm long, and such fragments were then carefully cultivated in culture at the new location, the risk of introducing associated macrobiota would be almost zero. The fragments would, however, still harbour a surface microbiota. Ideally it is desirable to translocate just a few cells of the plant and then grow these cells at the location using tissue culture methods. This was the procedure followed by Brazilian authorities when they introduced *Kappaphycus*. They imported axenic cultures, cultured the tissue in agar, grew plants, then grew a second generation of tissue cultures from those plants before releasing *Kappaphycus* into the sea. The process took about four years and required fairly sophisticated laboratory facilities (Oliveira and Paula 2003).

Like propagation by spores, tissue culture is unlikely to be a quarantine option in the developing Pacific Island countries, unless done in the regions Universities or research organisations, (eg USP, UPNG, University of Guam,
University of New Caledonia, the University of French Polynesia or IRD) or in a neighbouring country (eg. New Zealand or Australia) where such facilites are available. The various fisheries and agriculture departments in the region do not usually have the requisite facilities and often lack the necessary skills to produce the spores or tissue for export and to cultivate them at the new site.

<u>One way to reduce the volumes of cuttings that are imported to a new location is minimise mortality after transplanting</u>. If most transplants survive at the new site, then relatively few cuttings need to be imported in the first place, thus reducing the risk of bringing in associated species and hitchhikers. This may be achieved by creating a fenced nursery area to exclude large herbivores like rabbit fish. Routinely inspecting the plants to pick out smaller herbivores such as snails will also improve the survival and growth rates of the transplanted cuttings. So too

will taking care to selecting an appropriate transplant site. For example, if the cuttings come from an area where there is a moderate current and very clean water then they should be transplanted into a similar habitat, not a turbid area with little current.

From our experiments in maintaining *Kappaphycus* in non-circulating tanks of seawater we found that the condition, eg onset of chlorosis, of the transplants started to deteriorate within about two weeks. Declining concentrations of nutrients in a closed system, insufficient light (Suva was very cloudy at the time) and especially lack of any strong water movement are likely causes.

Seaweeds transferred from nutrient replete to nutrient depleted environments typically stay healthy for several days until their reserves of stored nutrients (usually N) are used up. Many red seaweeds store N as the water soluble pigment phycoerythrin. The pale colour of seaweed observed at the end of the quarantine period compared with their darker colour at the start lends support to the idea that tissue N levels had fallen to the point where growth was nitrogen limited during the second week.

If, however the seaweeds are kept in a seawater race-way system where there was a moderate current, then boundary layers (reducing absorption of nutrients) are broken down and the constant agitation results in cuttings that are more robust, and less likely to become covered with epiphytes. With a raceway system the quarantine period could be extended and probably the cuttings would continue to grow especially if selected nutrients were added.

What is needed is a closed system with a reservoir tank routinely topped up with new seawater, or a through-flow system where the “exhaust” water is discharged into a setting pond (which can be chlorinated and diluted with freshwater) followed by a sand filter or soak pit before reentering the sea. Neither of these options (closed or through-flow) is difficult to construct. Either will ensure a longer

quarantine period and an opportunity to “bulk up” the biomass of the transplants before they are placed out in the lagoon. Such improvements also reduce the volumes of seaweed that need to be imported, thereby reducing the risk of introducing unwanted species.

The proposed protocol does little to reduce the risk of introducing unwanted microorganisms with the *Kapapphycus* plants or cuttings. No amount of washing with filtered seawater will completely remove a surface film of diatoms, dinoflagellates and other microorganisms. Brief washing with filtered fresh water is, however, likely to be more effective because many surface-dwelling microbes are intolerant of low salinities, were as *Kappaphycus* can survive low salinities for short periods.

As mentioned in the General Introduction, transplanting *Kappaphycus* may also accidentally translocate ciquatoxic dinoflagellates. A way to reduce this risk is to disinfect the cuttings. Surface disinfecting of seaweeds is a very common cleansing step used by phycologists who are culturing seaweed spores and ova. The spore or egg-producing parts of the plants are typically soaked or wiped with a disinfectants such as Betadine™ or bleach to kill the surface microbes that would otherwise contaminate spores and eggs as they are released. If the seaweed is thoroughly rinsed afterwards, then it will survive the disinfectant.

It would be relatively simple, and desirable to include a disinfecting step to this protocol. This could involve soaking the *Kappaphycus* cuttings for short periods in solutions of copper sulphate at a concentration of about 30 ppt, which is good for killing fungi and other invertebrate parasites, and in Betadine™ or bleach solutions which is effective against a wide range of phytoplankton, protozoa and bacteria. Some experimentation is needed to test the tolerance of the cuttings to these disinfectants (copper sulphate is after all a powerful algicide). But once the the dose and length of immersion that will ensure that the cuttings still survive are known, then keeping the cuttings in quarantine is superfluous. Attention can then

shift to ensuring the rapid growth rate and bulking up of the transplants after the disinfecting step.

To conclude, we think that the washing protocol that we have followed is at best minimal. Several simple improvements could be made. Most important is to transplant the seaweed by way of cuttings taken from the tips of the plants, and to select for morphotypes that are relatively robust and streamlined. Then in addition to the washing procedure described by the protocol, to include a wash in fresh water. Thirdly we would recommend including a disinfection step because if that step is successful (ie it doesn't kill the *Kappaphycus* cuttings) then there is little need for any lengthy quarantine. These additional steps taken together with efforts to ensure a high survival rate of transplants will ensure that only small volumes of the seaweed need to be transplanted in the first place, and that the fragments that are transplanted are free of almost all associated epi-biota and microbes. Internal parasites such as fungi or viruses will however, be immune from such quarantine procedures.

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APPENDIX ONE

Detailed morphologies of *Kappaphycus alvarezii* from the different locations.

(1) Seaweed from Bua

(2) Seaweed from Savusavu

(2) Seaweed from Macuata

Revised SPC *Kappaphycus* Seaweed Quarantine Protocol

A. Pre-export

1. Seaweed propagules should be selected from the young healthy portions of plants that are free of epiphytic algae, injuries or necrosis (rotting tissue)
2. Small quantities of seaweed should be selected (10-20 kilograms) for shipment
3. The surface of propagules must be free of sediment, macro fauna and flora (i.e. any entangled drift seaweed or animals such as eels, worms or crustaceans)

B. Notification

1. The respective quarantine authorities of the importing country are to be notified in advance of transhipment, in accordance with the requirements of the particular national jurisdiction
2. Airline and freight agents are to be notified that the shipment contents contain live plant specimens, in accordance with their requirements

C. Quarantine facilities

1. Seawater supply is pre-treated by filtration through one micron filter cartridge
2. Where seawater source is oceanic, nutrient source such as NH_4Cl and KH_2PO_4 may be required. NH_4^+ concentration should be no more than 200 micro-molar and PO_4^- concentration should be no more than 20 micro-molar in the holding tanks. Where seawater source is lagoonal, addition of nutrient is not required.
3. Seawater salinity is at least 28 parts per thousand, and preferably 33-35ppt
4. Seawater temperature is stable and in the range of 25-30 ^{o}C
5. Aeration is provided to generate adequate water movement
6. The seaweed quarantine unit is isolated from other aquaculture facilities
7. Access to the quarantine facility is restricted to authorised personnel only
8. All other fauna or flora to be excluded from the quarantine facility
9. The seawater outflow is discharged into a sump pit which is out of range of the high tide water mark, at a location that can be safely treated with Chlorine
10. Equipment used in the quarantine facility, such as scrubbing brushes, thermometers, filters and etc, are to be treated with a chlorine dip after use
11. Holding tanks are to be drained and scrubbed clean at least twice a week

D. Treatment

1. Upon arrival the seaweed is to be soaked for one hour in one micron filtered fresh seawater. This is to allow recovery from stress during transportation.
2. After soaking in filtered fresh seawater, gently spray the seaweeds with freshwater (tap water or rainwater) to remove any microorganisms that may be loosely attached on the surface. Washing with freshwater should take no more than four minutes for each batch of seaweed (average weight 1 kg). *Kappaphycus* can survive short periods in low salinity; prolonged exposure beyond four minutes may result in the death of apical cells
3. Dip the seaweeds in filtered seawater for five minutes to allow recovery from the freshwater wash
4. Prepare a 2ppm copper sulphate solution (20 mg in 10 litres) in filtered fresh seawater `**IN ADVANCE' before arrival of seaweeds**. After the recovery treatment [D (3) above] dip the seaweeds in the copper sulphate solution for three minutes to act on any remaining invertebrates and other micro-algae
5. Rinse of the copper sulphate by dipping in filtered fresh seawater
6. The seaweeds should then be transferred to the holding tanks. Stock density per holding tank should be approximately one gram of seaweed per litre of filtered fresh seawater (1 g/L)
7. Seaweed stock is to be held under quarantine for nine days. Beyond nine days, the health of seaweeds begin to decline and further quarantine does not add much to risk management
8. Seawater in the holding tanks is to be changed after every two days
9. Discharged water [or any water used in washing seaweed eg D(1), D(3) and D(5) above] is treated with chlorine bleach for 24 hours at 125 ml m^{-3} dose before discharging into sump pit.
10. Stress of seaweed stock (e.g. drying in air) is to be minimized when cleaning during the quarantine period
11. Seaweed are to be visually examined daily for unusual signs
12. Seaweed samples are to be examined at random by surface microscopic examination using a magnifying glass (5x) for signs of epiphytes
13. A daily log is to be kept, recording details of treatment, observations and clinical abnormalities

E. Criteria for not releasing imported seaweed into the local environment

1. The presence of unexplained flora or fauna associated with the seaweed
2. Unexplained unusually high mortality of the seaweeds in quarantine
3. Unexplained lesions on the seaweed
4. Fungal infections on the seaweed
5. Suspicion that non-endemic organisms associated with the seaweed may be introduced into the wild

F. Ecological monitoring

1. Prior to out planting a baseline survey of species biodiversity is to be conducted within an area of 0.5 kilometres vicinity from the proposed farm site
2. Upon placement the seaweed are to be visually examined for abnormal signs of stress and mortality
3. The location of the seaweed is to be surveyed to see if the site is host to any unusual parasites
4. An area of 0.5 km vicinity surrounding the seaweed farm is to be monitored over a 1-year period for signs of unusual ecological disturbances or of loose seaweed becoming established in the wild in significant quantities.